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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. No. 168.

THE REVERSAL OF THE EFFECTIVE STROKE OF THE LABIAL CILIA OF SEA-ANEMONES BY ORGANIC SUBSTANCES.

BY G. H. PARKER.

IN a paper published a few months ago (Parker, :05), I called attention to the fact that the reversal of the effective stroke of the labial cilia of the sea-anemone Metridium marginatum, which takes place when a piece of crab-meat or fish-meat is placed on its lips, could be brought about by potassium ions. The facts, however, that the most effective solution in this respect, $\frac{5}{8}$ *m* NaCl containing $\frac{1}{8}$ *m* KCl, caused an excessive discharge of nettle capsules and an abnormal secretion of slime, and that the meat which induced reversal contained much less potassium than the weakest artificial solution that would bring about this change, led me to conclude that in the natural feeding of these animals some other materials than potassium ions must be the cause of the reversal. As I had previously tested the more likely inorganic substances and obtained negative results, except with potassium, I turned to the organic materials contained in meat, and my results in this direction are contained in the present paper.

For a number of the organic substances that I used and for other assistance during the progress of the work, I am under obligations to Dr. Otto Folin, of the McLean Hospital for the Insane, whose studies on metabolism had led him to prepare in a high degree of purity a

number of substances found in meat and important as reagents in the present undertaking.

In testing these substances, I usually pursued the following plan. Fresh sea-anemones were brought directly from the seashore, and experiments were carried out with them on the day on which they were collected and on the following day, *i. e.*, before the change of surroundings had materially influenced their ciliary reactions. In testing the various substances, a small piece of the best quality of chemical filter paper about one millimetre square was moistened in sea-water and placed on the lip of a given animal. In cutting and transferring such paper squares, care was taken that they should not become soiled by contact with the fingers or other sources of organic contamination. On being placed on the lips they almost invariably became caught in the mucus covering these parts and were swept outward by the underlying cilia. When such a piece of paper reached the outer edge of the lip, it was lifted off by the forceps with which it had been previously handled and kneaded on a clean glass plate with a small amount of the substance to be tested. In this way the substance, either as fine particles or as a partial solution, was well worked into the paper. The paper thus charged was next placed on the same spot on the lip from which it had been previously wafted outward and its course observed. After the paper had been discharged from the outer edge of the lip, or was well started down the œsophagus, it was picked off with forceps and the lips were thoroughly rinsed by squirting sea-water on them through a pipette. The animal was then allowed an interval of at least ten minutes in which to recover. For each substance tested four such trials were made on each animal at different parts of its lips, and four or five animals were used in each set of experiments. The following records indicate briefly the results of these trials.

Uric acid. — In these tests squares of filter paper, whether they contained uric acid or not, were invariably wafted outward over the lips, and thus this substance showed not the least evidence of reversing the ciliary stroke.

Creatinine. — Like uric acid, creatinine was ineffective as a ciliary reversing agent. This was somewhat surprising, for, as will be shown immediately, creatine is most vigorous in this respect. The creatine molecule ($C_4H_9N_3O_2$) differs chemically from that of creatinine ($C_4H_7N_3O$) only by one molecule of water, and yet the latter on repeated trials gave not the least evidence of

changing the ciliary stroke, whereas the former reversed it most efficiently.

Creatine. — As I have just stated, creatine reverses the effective stroke of the labial cilia with great regularity. Squares of filter paper which had been previously wafted outward, were moved when impregnated with creatine exactly as pieces of meat were, *i. e.*, when first put on the lip they moved slightly outward or remained motionless for a few seconds and then began slowly moving down the œsophagus where they disappeared into the interior of the animal.

To ascertain further particulars about the nature of this reaction, I prepared a solution of creatine by adding to a $\frac{5}{8}$ *m* solution of sodium chloride enough creatine to make a $\frac{m}{15}$ solution of this substance. From this stock solution two weaker solutions of creatine were made, a $\frac{m}{30}$ and a $\frac{m}{45}$, by adding appropriate quantities of a $\frac{5}{8}$ *m* solution of sodium chloride.

When a square of filter paper which has previously been discharged from the lips is soaked with the $\frac{m}{15}$ solution of creatine and is replaced on the lips, it behaves exactly as a piece of meat does, in that it first moves slightly outward, then irregularly, and finally inward. Further, when a piece of the ciliated lip of a sea-anemone is cut off and placed in sea-water under the microscope, the direction of its ciliary movement can be readily determined. If now the sea-water is withdrawn and the material is flooded with the artificial fluid containing $\frac{5}{8}$ *m* sodium chloride and $\frac{m}{15}$ creatine, ciliary reversal takes place within a minute or less and the reversed action continues as long as the tissue is covered with this fluid. On exchanging the fluid for ordinary sea-water, the normal direction of the ciliary stroke is quickly restored.

The reversal by creatine, unlike that produced by potassium ions, is unaccompanied by an excessive discharge of nettle capsules or by a special secretion of slime; in fact, reversal by means of this substance is in all respects like that produced by meat juice.

Results similar to those just described were obtained from the $\frac{m}{30}$ solution of creatine, except that filter paper soaked with this solution was not invariably swallowed and the solution itself induced reversal on pieces of the lip under the microscope only after about two minutes. In other words, this solution, though capable of reversing the stroke, was not so efficient in this respect as the $\frac{m}{15}$ solution.

All attempts to obtain reversal by applying filter-paper squares soaked in a $\frac{m}{45}$ solution of creatine or by immersing a piece of the lip

in such a solution, gave negative results, and I therefore conclude that a $\frac{m}{45}$ solution is too weak to induce reversal.

Since a $\frac{m}{30}$ solution will regularly reverse the ciliary stroke, and contains about the same proportion of creatine as has been found in vertebrate muscle, I believe that creatine is one of the effective materials in the reversal caused by fish-meat. Doubtless the potassium ions also present in the juices of such meat augment this action, though, as I have elsewhere stated (Parker, :05, p. 8), they are present in such meat in insufficient amounts to induce this action by themselves.

The opinion expressed by von Fürth (:03, p. 436) that creatine is a substance found only in vertebrates, though perhaps not absolutely beyond doubt, suggests that in crab-meat, which also quickly reverses the ciliary stroke, some other organic material effective in this respect, may be present; but on this question I have had no opportunity to make direct observations.

Other substances. — Since it seemed probable, from what has been stated in the preceding paragraph, that other organic materials than creatine were capable of reversing the ciliary stroke, I tried a few of the more available of these without reference to their occurrence in meat. No reversal was obtained from cane sugar, grape sugar, maltose, or sugar of milk (Patch's preparation) either when applied in solutions or in powders kneaded into filter-paper squares. Crystals of cane sugar when dropped on the lips slowly dissolve, but so long as an undissolved particle remains, it moves outward. This agrees with my earlier observations on this substance (Parker, '96, p. 115), and I conclude that the sugars probably play no part in the reversal of the ciliary stroke in Metridium.

With other food substances my experiences were different. Squares of filter paper which had previously been discharged from the lips of the sea-anemone were passed down its œsophagus, when they were impregnated with Witte's peptone, or with deuteroalbumose as prepared by Dr. Folin ('98, p. 156). In both these instances the reversal was as quick, and in other respects as normal, as with creatine. Asparaginic acid, which is by no means so soluble in sea-water as the peptone or the deuteroalbumose, also reverses the stroke, though sometimes the reaction is slow in appearing. These three cases, however, are of importance in showing that other organic substances than creatine may induce perfectly normal ciliary reversals, and suggest that the chemically complex mixtures which serve as

food for the sea-anemones probably contain numerous substances that might alone cause ciliary reversal, and thus aid in the feeding habits of these animals. I have already expressed my belief (Parker :05, p. 8) that the ingestion of pieces of india-rubber, which is accomplished in Metridium marginatum by ciliary reversal, is dependent on some soluble organic material in the rubber, and the fact that several organic substances have now been found to cause reversal, supports this opinion, though Torrey (:04, p. 210) has argued from what he has found in Sagartia davisi, that this reaction is dependent rather upon mechanical than chemical stimulation.

From the reactions to such food substances as peptone and deuteroalbumose, as well as from the reactions to creatine, I conclude that the reversal of the effective stroke of the labial cilia of Metridium in normal feeding is produced by the action of large organic molecules and not by ions, though, as previously stated, potassium ions, if sufficiently concentrated, will induce reversal.

Summary.

1. The effective stroke of the labial cilia in Metridium marginatum is not reversed by uric acid nor by creatinine, but is reversed by creatine.

2. A $\frac{5}{8}$ *m* solution of sodium chloride containing $\frac{m}{15}$ or $\frac{m}{30}$ of creatine will cause reversal, but one containing $\frac{m}{45}$ of creatine will not.

3. Since vertebrate muscle contains about the same proportion of creatine as the weaker reversing fluid, it is probable that creatine is an effective element in bringing about ciliary reversal when fish-meat is applied to the lips of Metridium.

4. No reversal was obtained with cane sugar, grape sugar, maltose, or sugar of milk.

5. Reversal was produced by Witte's peptone, deuteroalbumose, and asparaginic acid. In these three instances, as well as in the case of creatine, the reversal was unaccompanied by the excessive discharge of nettle capsules and of slime, and in other respects resembled the reversal produced in normal feeding.

6. The reversal in normal feeding is due chiefly to the chemical action of organic molecules, and not to ion action, though potassium ions, if sufficiently concentrated, will cause reversal.

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SOME NEW LABORATORY APPARATUS.

By S. P. BEEBE and B. H. BUXTON.

[*From the Department of Experimental Pathology, Cornell University Medical College, New York.*]

SEVERAL new pieces of apparatus have recently been installed in the laboratory of the Department of Experimental Pathology, and it has seemed worth while to give a brief description of some of them.

Automatic Compressed Air and Vacuum Apparatus.

This consists of two small pumps, quite similar in construction to bicycle pumps, driven by one-fourth horse power, 110 volt direct current motor. Each pump is connected to a 20-gallon iron tank from which the supply pipes are led to various parts of the laboratory. Each tank has an adjustable automatic switch through which the current supplying the motor is led. Since one motor runs both pumps, it is necessary to have the motor controlled by each tank independently. The automatic switch on the compression tank may be adjusted so that any desired pressure up to 40 pounds per square inch may be constantly available. We find a pressure of 20–25 pounds the most suitable.

The vacuum pump reduces the pressure to 60 millimetres of mercury. The compression tank is fitted with a safety-valve so that the pressure may not get too high in case one uses a large amount of vacuum. In actual practice it is seldom called into use. This apparatus has proved to be very convenient. The only attention it needs is to be oiled every second day. The uses to which compressed air may be put in the laboratory are various; the vacuum is used principally for filtering, particularly bacterial cultures, and in those laboratories that have, as this one does, a very low-water pressure, it is invaluable. The vacuum, however, is not sufficiently low for many distillation processes.

Centrifuge.

A centrifuge of high power is a necessity in a modern laboratory. Since we could not find one in the market which in price and speed answered our requirements, we have had one specially made.

The cut shows the general plan of the machine. A steel disc two feet in diameter and one-quarter of an inch in thickness has ten shallow slots cut in its periphery, and steel straps holding the tube trunnions are bolted firmly to the disc on each side of these slots. (The disc has recently been modified somewhat from the condition shown in the cut. Slots have been made for four large tubes instead of two as shown, and the straps holding the trunnions have been fastened to the disc by two bolts each. Reducing collars are provided so that the large trunnions may be used for the small tubes if desired.) The small tubes are of the same size as those usually found on the small hand centrifuges for urine analysis. The large ones hold 150 cubic centimetres each.

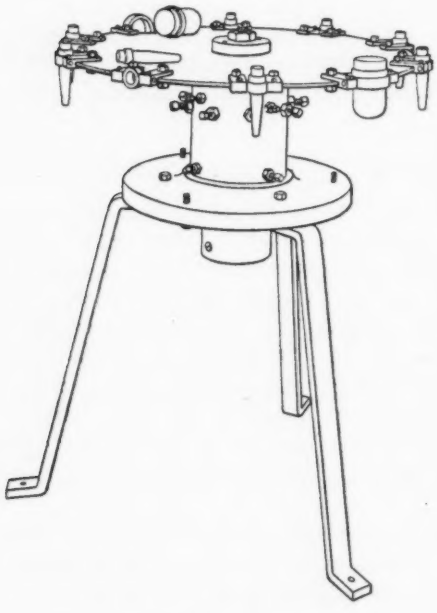

Figure 1.

The disc is mounted on a heavy steel shaft running in a ball bearing which is so constructed as to carry all the weight of the disc. The driving pulley is bolted to the lower end of this shaft. A belt of steel armor three-eighths of an inch in thickness surrounds the whirling plate. We have recently had a steel cover one-fourth of an inch in thickness made for the protective drum, and this is held in place by four screw clamps. These precautions are required because of the speed and weight of the moving parts. When

running at 3000 revolutions per minute, which is the usual speed, the large tubes exert a pull of 1500 kilograms and the small tubes a pull of 435 kilograms, the peripheral speed being 20,000 feet per minute.

The machine is operated by a direct current motor of 3 nominal horse power, but at our usual speed four horse power in current is actually required. Because of the high pressure (20 atmospheres) to which the glass tubes are subjected, it is necessary to float them in oil in the steel jacket tubes. For all of our work thus far with the large tubes we have used them without the inner glass tubes.

The work which this machine does is, however, of more interest than its mode of construction. Blood corpuscles are thrown down in a solid clump in two minutes, and to get a perfectly clear serum we need only to get the machine up to full speed and turn the current off almost immediately. With bacterial cultures a very clear fluid is obtained in eight minutes. We have made no tests to determine whether a sterile liquid could be obtained by prolonged centrifugation. The usual process of filtering through porcelain is a tedious one because of the plugging of the filter with the bacteria, but the centrifugated liquid obtained by eight minutes run at a speed of 3000 revolutions filters very readily, for practically all of the bacteria are left in a clump at the bottom of the tube and can be washed with but slight loss. If the bacteria have first been agglutinated by immune serum, an absolutely clear fluid is obtained in four minutes run.

This machine has been of great use to us also in working with proteids. Many proteid precipitates are filtered with extreme difficulty, and when one is dealing with a substance so easily injured and so liable to bacterial contamination it is of prime importance to finish the various operations of filtering and washing as rapidly as possible. The proteid precipitate is thrown down into a solid clump by this machine and the supernatant liquid poured off perfectly clear. We have found it particularly useful in preparing nucleoproteids from various glands. It has been possible to get out nucleohiston in a pure dry condition within twenty-four hours after the removal of the gland. In all such cases this machine not only saves a large amount of time, but enables one to get a much better product.

Drying Apparatus.

For many purposes the common method of drying proteids by means of alcohol is not suitable, and the practice of evaporating and drying in shallow plates in a vacuum or floating on a low-temperature

water bath is usually employed when one does not wish to injure the proteid. We have devised an apparatus which facilitates the drying of proteids at low temperatures.

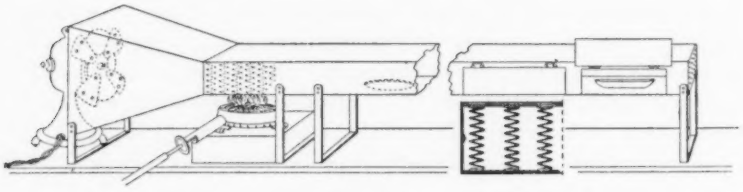

FIGURE 2.

The construction of the desiccator is shown by the diagram. A long shallow iron box has holes cut in the bottom to receive the drying plates. The electric fan drives a current of air through the box, and this current is warmed by passing through the heater, which is simply a portion of the box having a large number of copper strips passing from top to bottom. These strips are kept hot by the gas flame below. The temperature may be regulated either by the gas flame below the heater or by the speed of the air current. With the temperature at 40° C. the rate of evaporation is much more rapid than when the plates are floated on a water bath.

ELECTRIC WATER BATH.

The cut shows the principle of the apparatus, which is simple in the extreme. A 110 volt, 16 candle power incandescent lamp, with a small rheostat in the circuit, is surrounded by a cone-shaped double layer of thick felt. From above a beaker can be dropped in; the height of the cone being so arranged that when the flange of the beaker rests on the top of the cone its bottom just clears the lamp. A circular piece of asbestos, pierced so as to receive a thermometer, serves as a cover for the beaker. This apparatus has been found especially useful for inactivating serum at definite temperatures of 50°, 51°, etc. up to 60°. The beaker is partially filled with water, and warmed to two or three degrees above the desired point in an ordinary gas water bath. The tube containing the serum is then placed in the beaker, the temperature rapidly sinks, and as soon as it is a shade above that required, the beaker is dropped into the felt cone, which has previously warmed up by means of the electric lamp. For

temperatures below 60° C. the water bath must be constantly watched, and the light raised and lowered by changing the resistance with the rheostat. In this way the temperature can be kept within one-twentieth of a degree on either side of a desired point. For this particular line of work we had a special thermometer made, marked in fifths of a degree from 48° to 62° C.

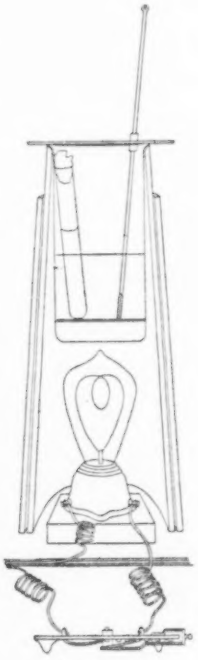

FIGURE 3.

The water bath can also be regulated for higher temperatures, even up to 90° C., and has been much used for sterilizing bacterial suspensions and other fluids at various degrees below boiling-point. For temperatures above 60° a little experience soon enables one to set the rheostat at the right point to maintain an even temperature, and the water bath can then almost be left to take care of itself. The chief advantage of this apparatus over the ordinary gas water bath is that the temperature can be much more accurately regulated with comparatively little care and attention.

We have found also that the heat from an incandescent lamp of 16 candle power is a very satisfactory means of boiling the ether in the ordinary fat extraction apparatus. There is no danger that the boiling will be too rapid, and in case of accident to the ether flask there is little danger of the vapor taking fire. It is much more convenient than the safety water bath made for gas burners.

SOME OBSERVATIONS ON THE CARBOHYDRATE METABOLISM IN PARTIALLY DEPANCREATED DOGS.

By PERCY W. COBB.

[*From the Physiological Laboratory of the Western Reserve University, Cleveland, Ohio.*]

THE following observations are offered as a contribution to the subject of carbohydrate metabolism in depancreated dogs:

The operation of pancreas extirpation was carried out after the technique of Witzel.[1] Four dogs were operated on. Two of these lived only a few days, three and seven days respectively. In the former the D : N ratio was over 3.0, and the autopsy showed a large duodenal ulcer. The latter gave a high D : N ratio, and the autopsy showed a good state of recovery as far as the site of operation was concerned, but extensive red hepatization involving parts of both lungs.

Another dog lived forty days from the time of operation, but the course of the diabetes was not typical, the urine containing less than one per cent of dextrose after the second day and no trace on the twelfth and the three days following. After this the estimations were discontinued. The fact that there was no typical diabetic condition makes this case of no interest in the present connection.

The remaining dog lived twenty or twenty-one days.[2] The results of nitrogen and sugar estimation are given in the table. The sugar estimation was made by Pavy's ammoniated copper method, and controlled by the polarimeter. The disagreement between the two seen in the results for the twelfth and sixteenth days of the experiment might be due to several causes, one of which could be the presence of beta-oxybutyric acid. On the other hand, acetone and aceto-acetic acid, the usual concomitants of that substance, were never definitely shown to be present.

[1] WITZEL: Archiv für die gesammte Physiologie, 1904, cvi, p. 173.

[2] As it died between Saturday and Monday, the exact number of days cannot be given.

Day of exp.	Day. Feb.	Food.	Urine + washings. c.c.	Nitrogen.		Dextrose.			D/N
				Per cent.	Total.	Polar. Per cent.	Red. Per cent.	Red. Total.	
1[1]	13–14	None	0	..	..	..	..	..	..
2	14–15	None	540	2.42	13.08	4.87	4.81	25.97	1.99
3	15–16	None	410	2.48	10.19	5.01	5.10	20.91	2.06
4[2]	16–17	None	300	2.67	8.00	5.69	5.88	17.64	2.20
5	17–18	None	372	1.91	7.12	3.92	4.07	15.14	2.13
6–7	18–20	None	983[3]	1.43	14.07[3]	2.70	2.82	27.72[3]	1.97
8	20–21	None	308	2.00	6.17	..	1.39	4.28	0.69
9–10[4]	21–23	None	500+[3]	1.53	7.66[3]	..	0.48	2.76[3]	0.31
11	23–24	None	320	1.02	3.25	..	0.24	0.77	0.24
12	24–25	24th, 200 gm. meat	296	1.50	4.45	0.88	1.22	3.61	0.81
13	25–26	25th, 200 gm. meat	162	3.10	5.02	..	3.76	6.09	1.21
14	26–27	26th, none	(?)[5]	2.32	..	..	1.14	..	0.49[5]
15	27–28	27th, 200 gm. meat	253	2.20	5.57	..	2.41	6.10	1.10
16	28–1 Mar.	28th, 200 gm. meat	243	2.26	5.49	1.68	2.19	5.32	0.97
17	1–2	1st, 200 gm. meat	233	2.12	4.93	2.67	2.43	5.66	1.15
18[6]	2–3	2d, 200 gm. meat	153	4.02	6.15	..	2.63	4.02	0.65
19[7]	3–4	3d, 50 gm. butter; meat refused	70	2.62	1.83	..	0.46	0.32	0.18
20–21[8]	4–6	4th, refused	90	1.35	1.21	..	0.00	0.00	0.00

Died between 4th and 6th. No sugar in specimen catheterized post mortem.

[1] Operation February 13.
[2] February 16, weight 7,700 gm.
[3] Totals for 2 days.
[4] No aceto-acetic acid.
[5] Catheterized specimen only was used. Pan soiled by fæces.
[6] No acetone, no aceto-acetic acid.
[7] Trace of acetone by iodoform test; none by ferric chloride test.
[8] Weight post mortem, 4,410 gm.

It will be seen that the D : N ratio was here never as high as 3, and that it decreased after the first six days almost to zero, increasing again when feeding (with lean meat) was begun.

As to the possible source of the sugar excreted a word is to be said. The total sugar recovered was 146.3 gm., equivalent to 131.7 gm. glycogen. The weight of the animal was 7.7 kg. If, then, the sugar was all derived from the body-glycogen, the latter must have been $\frac{131.7}{7.7}$ or 17 gm. per kg. body weight. This would be well within the maximum, which is given by Pflüger as 40 gm. per kg.,[1] and in view of the fact that the dog was in a good state of nutrition and had been starved but one day before the day of operation, it is reasonable to conclude that the body-glycogen was sufficient to account for all the sugar eliminated.

Against the body-glycogen as the source of all the sugar stands the fact that the feeding of meat caused a large increase in the sugar-excretion. For the twenty-four hours before feeding was started the total sugar excreted was 0.77 gm. If we subtract this from any subsequent daily amount, the remainder would represent the excess of sugar for that day, due to the feeding. Summing up these excesses gives 26.2 gm. dextrose excreted as the result of feeding 1200 gm. meat. If all this sugar came from the glycogen of the meat, it would imply that the meat contained 1.97 per cent glycogen. This is considerably above the normal percentage, and indicates some other source for the sugar excreted.

The autopsy disclosed a large abscess, bounded by the bend of the duodenum, the lobe of the liver above, and the duodenal mesentery below; from which about 10 c.c. of pus was obtained. The liver was tested for sugar by Pavy's alcohol-extraction method, and for glycogen by Pflüger's method, no trace of either being found. Fat was found in the liver to the extent of 3.64 per cent of the fresh liver, or 16.5 per cent of its solids.

A search for unremoved remainders of pancreas revealed what appeared to the eye to be a small piece of pancreatic tissue, triangular, lobulated, and measuring about $1 \times 1 \times \frac{1}{2}$ cm. Microscopically no alveolar structure could be seen in this piece of tissue. Sections of the mesenteric part of the duodenum were also made; microscopically, islands of tissue were found having a distinct alveolar structure; and in one instance the alveoli were seen to be filled with a structureless, apparently colloid material. The conclusion is that the extirpation was incomplete.

[1] *Vide* LÜTHJE: Archiv für die gesammte Physiologie, 1904, cvi, p. 160.

The feature of interest in this piece of work is the increase in absolute sugar-excretion, and as well in the D : N ratio resulting from the feeding of proteid. If we grant that the sugar is derived from the proteids of the body and of the food, this work adds weight to a view which has already been taken by Lüthje:[1] that the sugar from endogenous proteids is more readily oxidized by the tissues in the diabetic state than is the sugar derived from exogenous proteids.

[1] LÜTHJE: Münchener medicinische Wochenschrift, 1903, I, p. 1539.

COMPARATIVE PHYSIOLOGY OF THE INVERTEBRATE HEART. — PART III. PHYSIOLOGY OF THE CARDIAC NERVES IN MOLLUSCS (*continued*).

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I. The Cardiac Nerves of the Pulmonates.

SOME observations on the physiology of the cardiac apparatus with reference to the cardiac nerves have been made on the snail (Helix) by Foster and Dew-Smith, Ransom, and Yung.[1] Foster and Dew-Smith came to the conclusion that there are no nerve fibres in the substance of the heart of this mollusc, and they failed to find any nerve the stimulation of which in any way affected the heart. Ransom obtained the very opposite results. He not only found nerve fibres in the walls of the heart, but he showed by direct observation that stimulation of the left member of the pair of nerves which take their origin on the median protuberance of the pleuro-visceral ganglion arrests the auricle and the ventricle in diastole during the stimulation, and on cessation of the stimulation the rhythm reappears with increased rate and strength of beats. Ransom did not obtain any evidence of the presence of cardio-accelerator nerves.

My own work comprises three pulmonates, Ariolimax columbianus, Limax maximus, and Helix dupetitouarsi. The slugs, particularly Ariolimax, are very favorable for work along this line, because, thanks to their relatively large size, the visceral nerve is easily isolated and the ventricle is of sufficient size and strength to lend itself to graphic

[1] Foster and Dew-Smith: Proceedings of the Royal Society, 1875, xxiii, p. 318; Ransom: Journal of physiology, 1884, v, p. 261; Yung: Mémoires couronnées de l'académie royale de Belgique, 1888, xlix, p. 1.

registration. Even the delicate auricle may be made to lift a very light recording lever. Helix is much smaller than the two slugs, and in consequence the visceral nerve is shorter and the heart smaller and weaker; but in the largest specimens a length of nerve of about 3.5 cm. between the ganglion and the heart can be obtained, and by delicate manipulation both the ventricle and the auricle can be made to lift the recording lever.

The largest available specimens (about 20 cm. in length) of Ariolimax were chosen for the experiments. The following method of exposing the heart and the visceral nerve was found to be the most suitable. The slug was stretched out as much as possible without tearing the foot and the mantle and in this condition fixed to a board, ventral side down, by needles through the anterior and posterior ends. The dorsum was slit open on the right side near its union with the foot throughout the whole length of the animal, the foot fixed to the board by needles, and the dorsum turned over to the left and similarly secured to the board. In this way the whole viscera and the nervous system are exposed without severing any of its relations. The kidney is exposed from the ventral side, but as the heart is situated dorsal to the kidney, the left side of it has to be turned to the side and secured to the board by pins in order to expose the heart. The dorsal wall of the pericardium is slightly transparent, so that the movements of the heart may be observed through it; but the pericardium was usually slit open near its union with the kidney on the left side, even when the movements of the heart were studied by direct observation only, as this could be done without severing the cardiac nerves. The visceral nerve contains motor fibres to the musculature of the kidney, and it is therefore necessary to fix the kidney and the pallial complex to the board in such a way that the contraction of the kidney does not affect the movements of the heart. After isolating the visceral nerve down to its penetration of the kidney, the viscera were removed and the preparation ready for the experiments with direct observation.

When the visceral nerve is stimulated with a weak interrupted current, the rhythm of the beating ventricle is augmented or a rhythmical series of beats produced in the quiescent ventricle. This is the case whether the heart is left in the intact pericardial cavity and thus subjected to the pressure from the contraction of the kidney, or whether the pericardial cavity is laid open and the kidney pinned to the side so that its contraction does not affect the heart. It is further

more true of the ventricle after it has been separated from the auricle, so that variations in intracardiac pressure do not come into play. The effect on the auricular rhythm of stimulation of the visceral nerve is twofold, accelerator and inhibitory; and both these effects are obtained even after severing the ventricle, so that the accelerator and inhibitory nerves must enter the auricle at its junction with the venous sinus. The nerve to the ventricle enters by the aortic end, as severing the auricle does not impair the influence of the visceral nerve on the ventricle, but after severing the heart at the aortic end stimulation of the nerve seems to have no further effect on the ventricle.

For the graphic registration of the movements of the ventricle a silk ligature was secured to the heart at the auriculo-ventricular junction, and the board to which the preparation was fixed supported in a clamp, just as in the experiments of the heart of the abalone and the limpet.[1] From the way that the nerves reach the ventricle it ought to be possible to remove the ventricle from the kidney, save only a small fringe of the latter around the aorta in which the nerves pass to the ventricle, and suspend the ventricle by the aorta and the auricle, still retaining the ventricular nerves intact; but practically I found this very difficult to accomplish. The former method of suspension yields reliable results because nothing interferes with the movements of the ventricle and the preparation is readily made.

Typical tracings showing the augmentation of the ventricular rhythm on stimulation of the visceral nerve are given in Fig. 1. The tracing *A* is the most common form of the accelerator curve. The augmentor effects reach their maximum quickly and last but a few seconds, and are followed by decreased rate and strength of the beats even during the stimulation, the original rhythm returning gradually. In tracing *B* no such depressor after-effects appear in the rhythm; the augmentation reaches its maximum very gradually, and the cessation of the stimulation leaves the ventricle in improved rhythm as compared to that at the beginning of the stimulation. A relatively strong interrupted current is required to obtain this type of augmentation. By using an interrupted current of minimal strength in fresh and vigorous preparations the augmentation sometimes appears in increase of the rate without attendant change in the strength of the contraction or in the tonus (Fig. 1, *C*). In such cases there are no depressor after-effects of the slight acceleration.

In fresh preparations and with a strength of the interrupted current

[1] CARLSON: This journal, 1905, xiii, p. 396.

slightly above the minimal the augmentation was frequently followed by arrest of the ventricle in diastole for a few seconds, the prolonged pause in the rhythm being accompanied by a decrease in tonus (Fig. 2, *A*, *B*). This is obviously an extreme form of the decrease or inhibition of the rhythm that usually follows the augmentation (Fig. 1, *A*). As in the case of the ventricle and the ventricular nerve of Montereina, this inhibitory interference with the rhythm appears in some cases even when the preceding acceleration is very slight. In such instances it is difficult to believe that the marked decrease in the

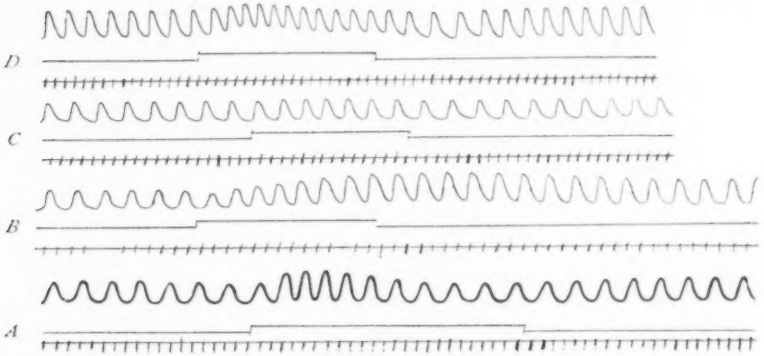

FIGURE 1. — Tracings from the ventricle of Ariolimax on stimulation of the visceral nerve. *A*, *B*, and *C*, interrupted current; *D*, constant current (2 Edison-Lalande cells). Showing augmentation of the rhythm.

height and rate of contraction is due to fatigue of the muscle from the previous slight augmentation, and one is led to consider the influence of possible cardio-inhibitory fibres in the visceral nerve. By comparing the tracings *A* and *B* (Fig. 2) it will be seen that the phenomenon is at least in one sense an after-effect of the stimulation of the nerve, for the continuation or the cessation of the stimulation appears to be indifferent to the appearance of the diastolic pause. If the interference with the rhythm is really due to the influence of inhibitory nerves, these inhibitory nerves must continue to send impulses to the ventricle for some time after the cessation of the stimulation of the nerve. This might readily be the case if the nerves were injured by the stimulation, but it is difficult to understand how the inhibitory fibres can be thus injured and not at the same time the accelerator fibres.

In three preparations out of thirty I obtained tracings showing

slight inhibition of the ventricular rhythm on stimulation of the visceral nerve with a weak interrupted current. One of these tracings is reproduced in Fig. 2, *C*. In this case the inhibition is manifested by decrease in the rate of the beats together with a slight tonus relaxation. The decrease in the strength of the contractions is very slight. The inhibitory effects are more readily obtained when the ventricle is isolated from the kidney, the pericardium, and the auricle in the usual way, but left in the pericardial cavity instead of suspended and weighted with the recording lever. As a rule the inhibition is obtained by a weaker strength of the stimulus than suffices to produce the augmentation; but in the majority of the preparations

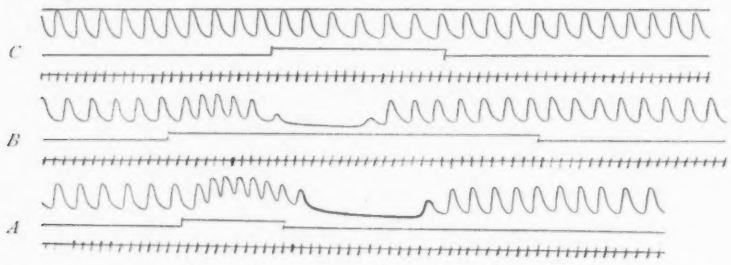

FIGURE 2. — Tracings from the ventricle of Ariolimax on stimulation of the visceral nerve. Heart severed at auriculo-ventricular junction. *C*, constant current; *A*, *B*, interrupted current. Showing possible inhibitory effects of the stimulation of the cardiac nerve.

even the most delicate graduation of the strength of the interrupted current failed to produce anything but augmentation of the ventricular rhythm. The fact that the inhibition is less readily obtained when the ventricle is subjected to the load of the lever may be due to the accelerator effects of the tension. Mechanical stretching of the ventricle is, within limits, a very efficient stimulus to rhythmic activity, and the effect of this stimulus has to be overcome in the suspended but not in the collapsed ventricle.

The records in Fig. 3 illustrate the effect on the ventricle of stimulating the visceral nerve with single induced shocks. Single shocks of the same strength as the interrupted current which produces distinct augmentation of the rhythm have no effect on the ventricle unless sent through the nerve at the rate of three or four per second, in which case the effect on the ventricle is the same as that of the interrupted current. Single shocks of somewhat greater intensity usually produce the typical acceleration and subsequent inhibition of the

rhythm even when applied to the nerve at as low a rate as one every other second (*B*); while if induction shocks of very great strength are employed, these effects may be produced by a single shock to the nerve (*A*). It is very probable that the nerve is injured by the induced shock of a strength sufficient to produce these effects.

The direct current from two Edison-Lalande cells usually produces augmentation of the rhythm for some seconds at the beginning of its application to the visceral nerve or the pleuro-visceral ganglion; but in four preparations distinct inhibitory effects on the ventricle were obtained. A typical tracing showing this reaction is reproduced in Fig. 1, *D*. The visceral nerve was stimulated near the ganglion, con-

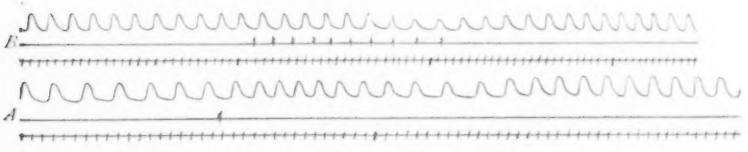

FIGURE 3. — Records from the ventricle of Ariolimax, showing the effects on the ventricle of stimulating the visceral nerve with single induced shocks. *A*, strong; *B*, weaker induced shocks.

sequently more than 4 cm. from the ventricle, by the current from two cells. The augmentation appears both in the rate and the strength of contractions.

The ventricular rhythm following the cessation of the stimulation of the nerve is in most cases the same as that preceding the stimulation; but in some instances the stimulation left the ventricle in a greatly improved rhythm.

It was stated that the visceral nerve has no influence on the ventricle after severing the heart at the ventriculo-aortic junction, but that after this operation its influence on the auricle remains the same as in the intact heart. This can be shown by the ordinary graphic method, the recording lever being connected to the heart by a silk ligature at the aortic end. A typical tracing from the combined auricle and ventricle obtained in this manner is reproduced in Fig. 4. The irregularities in the curves are caused by the lack of synchrony between the auricular and the ventricular beats. The co-ordination in the cardiac rhythm is always interfered with under conditions obtaining for taking the records, the auricle usually beating faster than the ventricle. This may be caused in part by the tension of the lever, which must be a more efficient stimulus for the auricle than

for the ventricle, as the walls of the auricle are much more delicate. By examining the myogram in Fig. 4 it will be seen that between each strong and apparently simple contraction are interposed two, but sometimes only one, weaker beat in which irregularities appear. The stronger and apparently single beats are the combined contractions of the auricle and the ventricle, and appear only when the systole and the diastole of the two organs coincide. The weaker and irregular beats are the results of lack of coincidence, the systole of the one being simultaneous with the diastole of the other. We shall presently learn that the most usual and potent influence of the visceral nerve on the auricle is inhibitory. This inhibitory action on the auricle and the absence of effect on the ventricle are clearly evi-

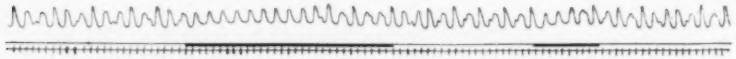

FIGURE 4. — Tracing showing the combined rhythm of the auricle and ventricle of Ariolimax, heart connected with the lever at the aortic end. Auricle and ventricle are beating with independent rhythms. Showing inhibition of the auricle and no effect on the ventricle by stimulation of the visceral nerve after severing the heart at its junction with the aorta.

dent in the myogram, for when the visceral nerve is stimulated with a weak interrupted current the irregularities in the curves disappear, but the main rhythm continues with unaltered rate, the beats not reaching the height of the apparent single contractions just referred to. The disappearance of the irregularities is due to the inhibition of the auricular rhythm. The irregularities reappear as soon as the auricle "escapes" from the inhibitory influence. The curves from the ventricular beats must of necessity be lower than the curves produced by the combined contractions of the auricle and the ventricle. On close examination of the ventricular rhythm during the stimulation of the nerve there will usually be found a gradual but slight increase in the strength of the beats at the beginning of the stimulation. This might be caused by an inhibitory influence that gradually loses its efficiency or by an accelerator influence of gradually increasing efficiency. But the phenomenon is not constant. The rate of the ventricular beats is not affected by the stimulation. In the tracing reproduced in Fig. 4 the rate prior to and during the stimulation is 30 per second; on cessation of the stimulation the rate is 31 per second.

To obtain tracings of the movements of the auricle alone, a silk

ligature was secured to the ventricle near its union with the auricle. The tying of the thread injures the ventricular muscle to such an extent that the small portion left in connection with the auricle makes no further contractions, while if the ligature is secured at the auriculo-ventricular junction the injury to the auricle is usually great enough to interfere with the rhythm. It was stated that the stimulation of the visceral nerve augments the rhythm both of the auricle and the ventricle in the intact heart. When the auricle has been subjected to the manipulations and conditions for graphic registration, the accelerator effects cannot always be obtained, the usual and the most marked effects being the inhibitory; but when augmentation of the rhythm follows stimulation of the nerve, the curves pro-

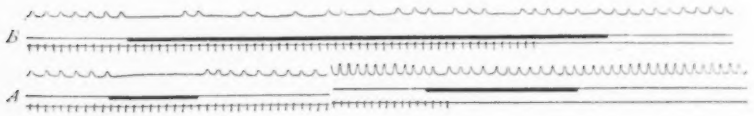

FIGURE 5. — Tracings from the auricle of ariolimax showing inhibition of the rhythm on stimulation of the visceral nerve. Auricle severed from the ventricle.

duced closely resemble those showing the accelerator effects on the ventricle. The augmentation of the auricular rhythm is sometimes preceded by a slight inhibition, and always followed by a decrease in the rate and strength of the beats for a few seconds prior to the return to the original rhythm.

Tracings illustrating the inhibitory effects on the auricular rhythm are reproduced in Fig. 5. The arrest of the auricle in diastole may be accompanied by a slight apparent increase in tonus; but much stress cannot be laid on this apparent tonus reaction, for the rise of the lever during the stimulation may be caused by the contraction of the kidney at the base of the auricle, as it is impossible to fix the kidney to the board so that the contraction of the portion immediately adjoining the base of the auricle is not communicated to the lever without severing the auricular nerves. When the inhibition is complete, the cessation of the stimulation is usually followed by an improved rhythm. Usually, however, the complete arrest of the beats lasts but a few seconds; but while the beats reappear, the conditions of the rhythm show plainly that the auricle is under the inhibitory influence for a much longer time or sometimes as long as the nerve is stimulated. The inhibitory influence appears in the rhythm

that prevails during the stimulation, either in reduced rate of the beats or in decrease both in pulse rate and strength of contraction. When the stimulation of the nerve with a weak interrupted current is long continued, the beats that appear during the stimulation sometimes show a grouping similar to that observed in the ventricle of Bulla on stimulation of the pleuro-visceral commissures.

Single induced shocks of the same intensity that proves efficient in the tetanic series do not appear to affect the auricle when applied to the nerve at a rate less than three or four per second.

The evidence obtained from the myograms thus supplements and corroborates that from direct observation: *the auricle is provided with both augmentor and inhibitory nerves; the ventricle is similarly provided with augmentor nerves and in all probability also with inhibitory nerves, but the influence of the augmentor nerves is predominant.*

The slug Limax is only about one-half the size of Ariolimax, but in the largest specimens the visceral nerve is large enough to be easily found and isolated, and it is sufficiently long (3 to 4 cm.) for the purposes of the experiments. In one respect this slug is more favorable for the work than Ariolimax, namely, in the small quantity of mucus secreted while being prepared. The great abundance of very sticky mucus excreted by the giant slug becomes a real hindrance both in the dissection and in the experiments. In Limax the mass of the kidney is dorsal and slightly posterior to the heart and the pericardium is transparent, so that when the dorsum is slit open and pinned to the side in the manner described for Ariolimax so as to expose the viscera, the action of the heart can be studied without laying open the pericardial cavity. When the visceral nerve in this preparation is severed from the pleuro-visceral ganglion, the ventricle ceases to beat and remains in diastole for several seconds. Stimulation of the severed or the intact nerve with the weak interrupted current has the same effect, namely, inhibition of the ventricle in diastole during the stimulation. On cessation of the stimulation the rhythm is resumed. The effect on the auricle is usually the opposite of that on the ventricle, the auricular rhythm is augmented. At the beginning of the stimulation interference in the auricular rhythm together with a diastolic pause of slightly longer than the normal duration sometimes appears, but the original or in most cases an augmented rhythm reappears in a few seconds.

When a strength of the interrupted current just sufficient to cause partial inhibition of the ventricular rhythm is employed, there

is usually no contraction of the kidney, but when a stronger stimulus is used the kidney contracts. This makes it difficult to decide how much of the disturbance of the cardiac rhythm is due to the influence of the cardiac nerves, and how much to changes in pressure brought about by the contraction of the kidney. That the change in pressure figures little, if any, in bringing about the change in the rhythm is shown by the fact that the effect on the heart of stimulation of the visceral nerve is the same after the pericardium has been slit open and the kidney pinned to the board so that its contraction does not produce any change in pressure on the heart.

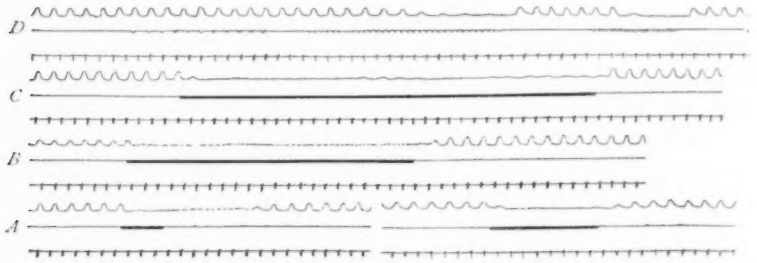

FIGURE 6. — Tracings from the ventricle of Limax on stimulation of the visceral nerve. Heart severed at the aortic junction. *D*, showing the accumulative effects of the single induced shocks to the nerve. The tracings show only the ventricular beats, as the auricle is too feeble to affect the lever.

The inhibitory effect on the ventricle is the same after severing the heart at the aortic end, but after severing the heart at the auriculo-ventricular junction, the aortic end remaining intact, or after severing the auricle at its base, the stimulation of the nerve has no further effect on the ventricle. The severing of the heart at the aortic end does not impair the influence of the nerve on the auricle.

The myograms reveal additional conditions of the rhythm as influenced by the stimulation of the visceral nerve. The contrivance for obtaining the graphic records was the same as for Ariolimax, only the lever had to be much lighter, as the strength of the heart is less. The tracings in Fig. 6 are from the ventricle left in connection with the auricle, but severed from the aorta, a preparation similar to that made use of in Ariolimax in obtaining the myogram in Fig. 4. The tracings from the Limax preparations show a perfectly regular rhythm, while the tracing from the Ariolimax preparation shows a fairly regular irregularity. The uniformity of the rhythm in

the preparations from Limax is not brought about by the coincidence of the auricular and ventricular systoles and diastoles, but by the inability of the auricular contractions to affect the lever, the auricle being too feeble.

With moderately strong stimulus the arrest of the ventricle is complete during the stimulation (Fig. 6, *A*). The cessation of the stimulation may be followed by an improved rhythm (*B*). The inhibition of the rhythm is accompanied by decrease in tonus, more evident on direct observation than in the tracings, because of the tension to which the delicate ventricle is subjected by the weight of the lever. In fact, in some of the records there appears to be an increase instead of a decrease of tonus in the ventricle during the stimulation of the nerve; but this rise of the lever is not due to a tonus contraction of the ventricle, but to contraction of the kidney to which the base of the auricle is attached; as I was not in every case successful in so fixing the kidney to the board that its contraction did not in any way affect the lever, and at the same time leaving the cardiac nerves unimpaired. The recovery of the ventricle from the inhibitory effects is gradual, that is, the effects last slightly longer than the stimulation; this is particularly the case when an interrupted current of great strength is used or if the preparation is fatigued. With a minimal stimulus the strength of the beats is greatly reduced, but the rate of the beats may not be appreciably altered (*C*). At first it appeared to me that the diminutive beats appearing on the nearly complete diastole might be those of the auricle, the arrest of the ventricle being complete, but on direct observation this is seen not to be the case, as diminutive beats clearly appear in the walls of the ventricle.

The accumulative effects on the ventricle of the stimulation of the visceral nerve are brought out in records like the one reproduced in Fig. 6, *D*. The position of the secondary coil remains unchanged, and when the tetanic series is sent through the nerve it is followed by prompt and complete arrest of the ventricular rhythm. When the shocks are applied to the nerve at the rate of one per second, the rhythm of the ventricle is not affected; when the shocks reach the nerve at the rate of two per second, the inhibitory effects appear six seconds after the beginning of the stimulation; while the inhibition following the same shocks applied to the nerve at the rate of three per second appears almost as quickly as when the tetanic series is made use of.

During the course of an experiment it was frequently found that after repeated stimulations of the visceral nerve with the interrupted current, the action of the nerve on the auricle was much lessened or completely abolished, while its influence on the ventricle was but slightly impaired. This peculiar condition is of frequent occurrence. It may be that the nervous mechanism to the auricle is more readily fatigued, or its control of the organ less complete than that of the ventricular nervous mechanism; or the simultaneous stimulation of the two antagonistic sets of nerve fibres to the auricle may, under some conditions not yet understood, tend to counteract each other, so that their influence would not be evident, while the stimulation of the one kind of nerves to the ventricle produces the usual inhibition of the rhythm.

The absence of effect, inhibitory as well as accelerator, on the ventricular rhythm of stimulation of the visceral nerve after the heart has been severed at the auriculo-ventricular junction has already been noted. In view of the fact that in Ariolimax the main, if not the sole, nervous supply to the ventricle reaches the ventricle at the aortic end, one would expect the same relation to obtain in the closely related Limax; but that is, according to all evidence, not the case.

These experiments make it evident that *the auricle of Limax is provided with both augmentor and inhibitory nerves, and that the ventricle is supplied with inhibitory nerves, which enter it through the walls of the auricle.* The difference that thus obtains between the innervation of the ventricle in the two slugs appears to me too great to be actual in the case of two animals so closely related. There is no doubt of the existence in Ariolimax of accelerator fibres to the ventricle, entering it at the aortic end, and we have seen that there is some evidence that inhibitory fibres reach the ventricle in the same way; while in Limax there is no doubt of the presence of inhibitory nerves, which appear to reach their destination solely through the auricular walls. Despite my failure to find accelerator fibres to the ventricle of Limax, I would not be surprised if further researches should reveal their presence.

Two species of Helix were available for the work. Both are of smaller size than the slug Limax, and for that reason less suited for the experiments, as the visceral nerve is relatively short and not so readily isolated. The heart is smaller than that of Limax, but the ventricle is strong enough to register its contractions graphically

without any trouble, and I even succeeded in obtaining ordinary myograms from the auricle, although it is less than half the size and strength of the ventricle. There is less difference in size and strength between the auricle and the ventricle in Helix than in the two slugs, and the auricle of Helix is more muscular and actually stronger than the auricle of Limax. The snail was taken up with the view of testing the observations of Ransom (1884) on the heart and heart nerves of the same genus; and also with the hope that the cardiac nervous mechanism of this pulmonate might afford some clue to the apparent discrepancies found in the two slugs.

The heart of Helix is placed dorsal to the visceral mass and the left of the kidney. The pericardium and the visceral envelope dorsal to the heart are transparent, so that after removal of the shell the cardiac rhythm can be observed without previous dissection in the region of the heart. Foster and Dew-Smith (1875) state that the slitting open the pericardium and exposing the heart has a decidedly injurious effect on the rhythm. I did not find this to be the case. If care was taken not to pull or tear the heart or touch it with the instruments, the cardiac rhythm was neither accelerated nor retarded by the operation; but if the heart was injured in any way, irregular rhythm or cessation of the beats accompanied by tonus contraction would follow. When the heart is exposed but left intact in the pericardial cavity, the rate of the beats usually does not exceed 15 per minute. The heart is, of course, empty and collapsed, as the removal of the shell and the dissection of the visceral nerve involve extensive bleeding. When the visceral nerve in this preparation is stimulated with a weak interrupted current, the effect on the heart is complete cessation of the rhythm, both the auricle and the ventricle remaining in diastole during the stimulation. There is usually no distinct augmentation of the rhythm on the cessation of the stimulation. Severance of the auricle does not impair the effects on the ventricle of the stimulation of the nerve, nor does severance of the ventricle impair the action of the nerve on the auricle. It is therefore evident that the nerves enter the ventricle at the aortic end and the auricle at its base. If a weak stimulus is employed, these effects on the cardiac rhythm can be observed without any attendant contraction of the kidney and consequent displacement of the pericardial cavity and the heart; but when the visceral nerve is stimulated with a relatively strong interrupted current, the kidney and the lung-sac contract strongly. The interference with the cardiac rhythm is, however, not caused by the contraction of

these organs, because the inhibition is just as marked when the pericardium has been slit open and the kidney and the lung pinned to the board in such a way that their contraction does not affect the heart. These results are in perfect accord with those of Ransom: the auricle and the ventricle of this pulmonate are undoubtedly supplied with inhibitory fibres from the visceral nerve.

When a silk ligature is secured to the auricle near its union with the ventricle, the auricle severed at its base, and the ventricle connected with a light recording lever in the manner described for the slugs, the rhythm of the ventricle is immediately improved. Ventricles beating with a rate of 10 to 15 per minute when lying in the

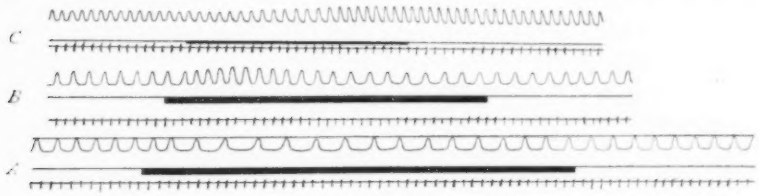

FIGURE 7. — Records from the ventricle of Helix on stimulation of the visceral nerve. Heart severed at the auriculo-ventricular junction. Showing both inhibition and augmentation of the rhythm. Interrupted current.

pericardial cavity will beat with a rate of 50 to 60 per minute when suspended in this manner. The difference in the rate can only be due to the tension of the lever. If protected from evaporation the suspended ventricle keeps up a regular rhythm for several hours.

Stimulation of the visceral nerve in the suspended preparation brings out both inhibitory and accelerator effects on the auricular as well as on the ventricular rhythm. The inhibitory influence on the ventricle is illustrated in tracing *A*, Fig. 7. The most usual form of the curve is a complete arrest of the ventricle in diastole for a few seconds at the beginning of the stimulation without any change in the tonus. In the suspended ventricles I never succeeded in obtaining complete arrest of the rhythm in diastole for more than a few seconds, while in the intact heart lying collapsed in the pericardial cavity complete inhibition may be obtained for from thirty to sixty seconds. The lessened efficiency of the inhibitory influence on the suspended ventricle is evidently due in part to the tension on the ventricle which acts as a constant stimulus, and also to the fact that severing the auricles cuts off some inhibitory fibres which, as we shall presently

learn, reach the ventricle through the auricular walls. The inhibitory impulses reaching the ventricle are consequently decreased, and this decreased inhibitory influence has to overcome the automatic activity of the heart tissue augmented by the powerful accelerator stimulation from the tension. The inhibitory effects are almost invariably greatest at the beginning of the stimulation. But frequently the inhibition appeared, not in a prolonged diastolic pause, but in decreased rate and strength of the beats. In some cases the decrease in the rate is very marked, while the decrease in the strength of the beats is hardly perceptible; in other cases the decrease in the strength of contraction is more pronounced than the decrease in the rate. The inhibitory effects thus far described have been obtained by stimulation of the nerve with the interrupted current. Slight inhibition of the ventricular rhythm was sometimes obtained during the passage of the direct current (two cells) through the nerve.

The accelerator effects on the ventricle of stimulation of the nerve were less frequently observed than the inhibitory effects, and it usually required a slightly stronger stimulus to obtain the augmentation. The usual form of the myograms showing the augmentation of the ventricular rhythm may be gathered from Fig. 7, *B*. The augmentation appears both in the rate and in the strength of the beats, and it is most marked at the beginning of the stimulation. The acceleration is usually followed by a transient decrease in the rhythm as compared to that preceding the stimulation. In tracings like that reproduced in Fig. 7, *C*, there seems to be a combination of the two effects, a slight initial inhibition being followed by a marked increase in the strength of the beats. In two preparations augmentation of the rhythm was obtained during the passage of the direct current through the nerve. These results make it evident that *the visceral nerve sends both inhibitory and accelerator fibres to the ventricle by the aortic end, the influence of the inhibitory fibres being the greatest.*

In order to determine whether any augmentor or inhibitory fibres reach the ventricle through the auricular walls, the heart was prepared and attached to the recording lever by a ligature at the aortic end, just as in the slugs. If the lever was adjusted so that its weight was the same or only slightly less than in the experiments on the ventricle alone, the auricular contractions hardly affected the lever; but the auricle was able to lift the lever if it was made so light that its weight was just sufficient to overcome the friction of the writing point against the smoked paper. Under the conditions of the experi-

ments there is no co-ordination between the auricular and the ventricular beats, in consequence of which the tracings show an apparently irregular rhythm, similar to that of the auriculo-ventricular preparation of the slug (Fig. 4).

This auriculo-ventricular preparation of Helix sometimes manifested a peculiar irregularity in the rhythm that at first appeared to be a periodic variation in the tonus of the muscle. A typical tracing of this kind is reproduced in Fig. 8, *A*. With the fairly constant rate and strength of the beats is coupled a series of long undulations of the lever in periods of about ten seconds' duration. The regular pulsations represent the ventricular beats, and the long undulations are caused by the movements of the auricle. This is evident from direct

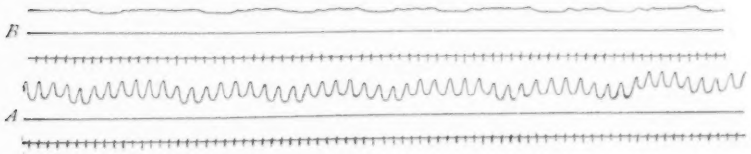

FIGURE 8. — *A*, tracing from auricle and ventricle of Helix, showing a peculiar secondary rhythm due to tension on the auricle. *B*, grouping of beats or tonus rhythm of auricle of Helix due to tension.

observation. These movements of the auricle do not represent single beats, nor are they tonus contractions; they are, in fact, periods of very rapid but diminutive beats separated by prolonged diastolic pauses during which the auricle relaxes more than during the normal diastole. Under certain conditions the auricle, separated from the ventricle, beats with this peculiar rhythm, as will be seen by referring to Fig. 8, *B*. The rapid diminutive beats can in these cases be made out even on the tracings, despite the fact that the lever diminishes the height of contraction. It will be readily seen that superimposing the ventricular rhythm on the curve in Fig. 8, *B*, we would obtain a record similar to *A*.

Stimulation of the visceral nerve in this preparation inhibits the ventricular rhythm, just as when the ventricle was left in connection with the aorta, but the effects are less marked. No undoubted accelerator effects on the ventricle were ever obtained on stimulation of the nerve with the heart in this condition. There is sometimes a slight indication of an increase in the strength of the beats, but the difference is not sufficiently marked to be conclusive. There is,

however, no doubt about the inhibitory effects; and it is therefore certain that *some inhibitory fibres reach the ventricle also by the way of the auricle.*

We will now turn our attention to the auricular nerves. It has already been stated that the lightest lever practicable had to be used for the experiments on the auricle; but even this slight weight was sufficient to keep the auricle abnormally extended and interfere with its rhythm, except in the case of auricles from the largest specimens. The ligature by which the lever was attached was secured to the ventricle near its union with the auricle. The small portion of the ventricle that thus remained attached to the auricle was so injured by the ligature that it gave no further contractions, and consequently does not figure in the tracings, while if the ligature was secured at the auriculo-ventricular junction, the auricle usually received sufficient injuries to seriously interfere with its rhythm. But with the thread tied to the auricular end of the ventricle, and with a light lever, the auricle would keep up a perfectly regular rhythm for two to three hours, the usual rate of the beats being about 45 per minute. Auricles that accidentally received slight injuries in preparation or auricles from smaller individuals for which even the lightest lever was too heavy, would sometimes exhibit no automatic activity, but some of these preparations maintained an irregular rhythm with more or less definite grouping of the beats. The preparations showing these irregularities were not used for the study of the function of the auricular nerves.

The influence of the auricular nerves is twofold: inhibitory and augmentor. Typical tracings showing the inhibitory effects are given in Fig. 9, *A* and *B*. As a rule the inhibition is greatest at the beginning of the stimulation, and the beats that appear during the stimulation are of less frequency and strength than in the original rhythm. Cessation of the stimulation is marked by the return of the auricle to the original rhythm. The inhibition of the rhythm is not accompanied by any change in tonus. In some tracings there appears, to be sure, a slight tonus relaxation, in others again a slight tonus contraction; but these exceptional reactions are in all probability due, not to any change in the tonus of the auricle, but to the contraction of the kidney and the lung to which the kidney is attached, as these could not in every case be so securely fixed to the board that their contraction did not affect the lever. In view of the influence of the inhibitory nerves on the cardio-tonus of other

molluscs, it seems quite certain that these nerves produce decrease of the tonus also in the auricle of the snail. That the tonus relaxation does not appear in the tracings is probably due to the fact that the muscle fibres are already abnormally extended by the tension from the weight of the lever. A not uncommon form of the myograms is represented in *B*. In these myograms the initial inhibition is followed by a return of the auricle to the original, or possibly a slightly augmented rhythm, for a few seconds, when the inhibitory influence again becomes apparent. Similar reactions have already been described for the auricle of Ariolimax, which is also supplied

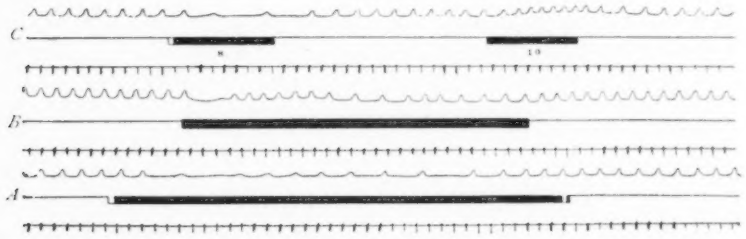

FIGURE 9. — Tracings from the auricle of Helix on stimulation of the visceral nerve, showing both inhibition and augmentation. In *C*, inhibition is produced by the interrupted current of 8 units strength, augmentation by the current of 10 units.

with nerves of antagonistic functions; and in the ventricle of Bulla, to which only accelerator nerves could be found.

The accumulative effects of the inhibitory nervous impulses are similar to those already described in the slugs. Single induced shocks of moderate intensity must be applied to the nerve at a rate not less than one per second to affect the auricle. When stronger shocks are used, they may come further apart and still be effective, but it is probable that the nerve is injured by the induced current of that strength.

The inhibitory effects are the most marked, and the first to be obtained when the strength of the stimulus is gradually increased from zero to a sufficient intensity to excite the nerve. But if the strength of the stimulus is slightly increased above this point, either acceleration or inhibition of the rhythm may be produced. This is shown in Fig. 9, *C*. At the left-hand side the nerve is stimulated with the interrupted current of the strength of eight units, and the auricle is arrested in diastole; when the strength of the stimulus is increased to ten units, the auricular rhythm is augmented.

The acceleration appears more in the rate than in the strength of the beats. The fact that as a rule a slightly stronger stimulus is required to produce the acceleration suggests that these results might be due to escape of the current directly on to the auricle, but from various checks and tests I am satisfied that such is not the case. The electrodes were always placed on the isolated nerve close to the pleuro-visceral ganglion, and were thus always at a distance of 2.5 to 3 cm. from the auricle. The strength of the interrupted current that produces acceleration is hardly felt on the tongue. When the visceral nerve is severed near the heart, the central end remaining on the electrodes, and the peripheral end placed near the base of the auricle, an increase in the strength of the stimulus up to five hundred units fails to affect the auricle, while with the nerve intact an intensity of from ten to fifteen units suffices to produce the augmentation.

The direct current from one Edison-Lalande cell produces inhibition of the auricular rhythm during its passage through the nerve. Accelerator effects were rarely produced by the direct current, and in the few cases where the tracings show an augmentation of the rhythm, the augmentation is usually preceded by a brief inhibition of the rhythm.

The cardio-inhibitory fibres in the visceral nerve of Helix are readily excited by all kinds of stimuli: sectioning, crushing, heating or drying of the nerve produces definite inhibitory effects, especially on the auricle. In a few preparations the mere placing the nerve on the platinum electrodes produced slight inhibition of the auricular rhythm. That this reaction was due to local action at the point of contact of the nerve with the metal seems to be shown by the fact that it remained the same when the wires from the electrodes were disconnected so that there was no possibility of the presence of any current in the electrodes. The inhibition never amounted to complete arrest of the auricle in diastole. The effect was always most marked immediately after placing the nerve on the electrodes, and usually subsided in ten to twenty seconds. The nerve was always handled by means of a silk thread tied to the nerve near the ganglion, so that the excitation could hardly be caused by the manipulation. Nor do I think that evaporation or drying of the nerve played any part. But further studies are required to determine whether the excitation is the result of chemical action, or of the making of a circuit for the current of injury in the nerve.

The auricle of Helix is extremely sensitive to mechanical stimula-

tion, and such stimulation always accelerates the rhythm, unless sufficiently violent to injure the auricle, in which case there is a great increase in the tonus of the auricle coupled with a complete absence of beats, or at most only a few irregular and diminutive beats. It is, of course, possible that what appears to be a tonus contraction is in reality a tetanus contraction. Arrest of the rhythm in diastole was never obtained by direct mechanical stimulation.

These results obtained by the graphic method thus confirm the observations of Ransom as far as they go. *The auricle and the ventricle of Helix are supplied both with accelerator and inhibitory fibres from the visceral nerve. Inhibitory and accelerator fibres enter the ventricle at the aortic end, and some inhibitory fibres reach the ventricle through the auricle. The nerves to the auricle enter at the base of that organ. The inhibitory fibres are most readily excited, and their influence on the heart most marked.*

In comparing the results obtained in these three pulmonates, the following points may be noted: (1) There is no difference in the innervation of the auricle: both accelerator and inhibitory fibres from the visceral nerve entering that organ at its base. (2) Singular discrepancies seem to obtain in the innervation of the ventricle. In Ariolimax the fibres enter solely by the aortic end, in Limax solely through the walls of the auricle, while in Helix nerve fibres enter the ventricle both by the aortic and the auricular ends. Similar discrepancies appear in the function of the ventricular nerves. In Ariolimax the accelerator nerves are paramount, with a mere indication of the presence of inhibitory fibres; in Limax the ventricular nerves appear to be wholly inhibitory; while in Helix there are plainly both accelerator and inhibitory fibres, the influence of the latter being in preponderance. But I would not go to the extent of claiming that the absence of accelerator nerves to the ventricle of Limax is proved by my negative results. (3) When we compare the results in the pulmonates with those obtained in the marine gasteropods, even greater differences come to light. In the lower gasteropods (prosobranchs, tectibranchs) the cardiac nerves are uniformly accelerator, and apparently only accelerator; at least in one representative (Triopha) of the nudibranchs there is evidence of cardio-inhibitory fibres, besides the accelerator fibres; while in the highest gasteropods (Helix, Limax) the inhibitory function of the cardiac nerves predominates over the accelerator function.

II. The Cardiac Nerves of the Cephalopods.

Observations on the physiology of the cardiac nerves in the cephalopod molluscs have been made in Sepia by Bert (1867), and in Octopus by Fredericq (1878), Ransom (1884), and by Bottazzi and Enriques (1901).[1] Bert found that stimulation of the visceral nerves caused inhibition of the systemic heart. The same results were obtained by Fredericq on Octopus. Ransom, who studied the influence of the visceral nerves on the systemic heart of Octopus both by direct observation and by the graphic method, sums up his results as follows: "The action of the visceral nerves is to cause ordinary inhibition of the ventricle and of the auricle of its own side, but to provoke the opposite effect of contraction in the branchial heart and the gill of that side." The results of Bottazzi and Enriques touching these points are to the same effect, namely, that the visceral nerves send inhibitory fibres to the auricles and the systemic ventricle, and accelerator fibres to the gill ventricles. Neither Ransom nor Bottazzi and Enriques are able to affirm anything definite as to the innervation of the vena cava and the renal veins. Fuchs[2] has studied the influence of the visceral nerves on the vascular apparatus of Octopus by recording the blood pressure by means of a cannula in the cephalic aorta near the heart. He found that the stimulation of the visceral nerves causes great increase in blood pressure coupled with more or less complete inhibition of the cardiac rhythm. He refers this increase in the blood pressure to the influence of vaso-motor nerves, claiming that the change in the blood pressure cannot be ascribed to contraction of muscles other than those of the blood vessels, because in the majority of cases the stimulation produced no movements of the animal. But he had the animal placed on its ventral side, and could therefore not observe the displacement of the viscera and the extreme contraction of the muscular septa that connect the peri-visceral sac with the mantle, which invariably follow the stimulation of the nerves. The visceral nerves contain in addition motor fibres to the gills and the musculature of the visceral envelope, and it is

[1] Bert: Mémoire de société scientifique de Bordeaux, 1867, v; Fredericq: Archives de zoologie expérimentale, 1878, vii, p. 535; Ransom: Journal of physiology, 1884, v, p. 261; Bottazzi and Enriques: Archives italiennes de biologie, 1901, xxxiv, p. 111.

[2] Fuchs: Archiv für die gesammte Physiologie, 1895, lx, p. 173.

obviously impossible to determine how far the change in the blood pressure is due to contraction of the musculature in the walls of the blood vessels and how far due to the pressure on the cardiac apparatus and the blood vessels exerted by the contraction of muscles that do not form a part of the vascular apparatus.

We will first consider the conditions obtaining in the squid (Loligo). After the mantle has been slit open lengthwise in the ventral median line, the animal placed on its back in a dish of sea-water, and the edges of the mantle fixed to either side so as to expose the viscera, the respiratory movements of the mantle and the siphon continue for five to ten minutes, but the cardiac apparatus does not maintain its co-ordination. The gill ventricles and the systemic ventricle are sometimes found in diastolic rest immediately on opening the mantle cavity, in which case pulsations usually begin on mechanical stimulation of the ventricles or on severing the visceral nerves. The beats commence without resorting to any of these stimuli, if the preparation is left undisturbed for fifteen to twenty seconds. But more often the renal veins and the gill ventricles are pulsating at the rate of 60 to 80 per minute, the systemic ventricle beating very irregularly and in most cases at the rate of only 8 to 12 per minute. In the preparations in which the rhythm of the systemic ventricle was more regular, the rate was always less than one-third of that of the gill ventricles, and the beats appeared to come in the respiratory pause. The auricles beat with greater regularity and faster than the systemic ventricle, but not so fast as the gill ventricles. In preparations in which the rhythm of the systemic ventricle was very irregular or had ceased entirely, a reversal of the contraction wave in the efferent gill sinuses was sometimes observed; that is, the contraction started at the base of the gills and travelled towards the apex instead of starting at the apex and travelling towards the base and the auricles in the normal way. The pulsations of the cephalic vena cava appear to be synchronous with the respiratory movements, but they will generally continue for some little time after the respiratory movements have ceased. The anterior part of the vein continues to beat after the posterior part has ceased to contract, and when the whole vein is pulsating, cross-section of the vein usually stops the pulsations of the posterior part, the anterior end of the vein continuing its contractions. *The vein ceases to beat when the pleuro-visceral ganglia are destroyed.*

The complete loss of co-ordination of the cardiac apparatus in lay-

ing open the mantle cavity is probably not due to defective oxidation of the blood in the gills, for placing the preparation in running sea-water does not alter the conditions. The mere slitting open of the mantle does not cause so extensive bleeding that the interference in the rhythm could be ascribed to the change in internal pressure. The influence of the nervous system, together with the change of pressure in the mantle cavity, is perhaps the essential factor in bringing about the loss of co-ordination. No less extensive operation suffices to expose the whole cardiac apparatus, and I was therefore never able to observe the normal rhythm and sequence of the different parts.

The systemic ventricle ceases to beat in four to five minutes, the auricles continue somewhat longer, the renal veins and the gill ventricles generally pulsate with gradually decreasing rate for twenty to thirty minutes. The respiratory movements cease in a few minutes, particularly if the preparation is taken out of the sea-water; a preparation in which the respiratory movements have ceased will often resume these movements if placed in sea-water. The activity of the respiratory centre is therefore probably dependent on afferent impulses from the mantle and the gills. The respiration goes on after removal of the optic and the cerebral ganglia. In three out of fifty preparations severance of the visceral nerves stopped the respiratory movements, and in these three preparations the movements were resumed after some ten to twenty seconds' stand-still.

When in this preparation both of the visceral nerves are dissected out, severed from their central connection, lifted out of the water and stimulated with a weak interrupted current, the cardiac apparatus and the whole visceral mass are compressed and displaced by the contraction of intervening muscular septa and the viscero-pericardial sac, which closely invests the auricles, the renal veins, and the gill ventricles. The systemic ventricle, lying deeper in the visceral mass, suffers less actual compression, but it is displaced together with the rest of the viscera. If the auricles and the systemic ventricle are pulsating, the rhythm ceases and the organs remain in diastole during the stimulation in case the interrupted current is relatively strong. With a weaker stimulus complete inhibition is obtained for twenty to thirty seconds only. On cessation of the stimulation the ventricle and the auricles begin to beat with a greater strength and rate than prior to the stimulation.

The renal veins are partly contracted or rather passively com-

pressed. In some cases they beat slowly and irregularly during the stimulation, in other cases there is a complete arrest of the rhythm at the beginning of the stimulation, but irregular and feeble beats soon appear. The gill ventricles appear partly contracted, sometimes giving a few irregular beats during the stimulation; in few preparations actual acceleration of the rhythm was produced. On cessation of the stimulation the rhythm is resumed, usually with an augmented rate and strength of the beats. The gills remain in extreme contraction during the stimulation.

When only one nerve is stimulated the effect on the systemic ventricle is the same as when both nerves are stimulated, and the effect on the auricle, the gill ventricle, and the gill of the side of the stimulated nerve also the same; but the gill on the opposite side is not contracted, the gill ventricle beats sometimes slower and sometimes faster, and the auricle appears to be partly inhibited. The effects on the renal veins appear to be the same as when both nerves are stimulated.

It is obvious that these conditions do not allow accurate determination of the influence of the visceral nerves on the cardiac muscle. For that purpose the displacement, compression, and tension on the heart must be excluded. This is most readily accomplished in the systemic ventricle. By careful dissection the renal veins can be removed from the ventral surface of the ventricle, the attachments to other parts of the viscera severed, and the auricles severed close to the base of the gills, leaving the nervous connection intact. The ventricle can now be experimented on *in situ* or removed from the body together with the visceral nerves and suspended by the anterior and the posterior aortæ for graphic registration. Usually but one of the nerves was dissected out because the dissection requires some little time, the nerves die quickly, and the heart ceases to beat soon after opening the mantle cavity.

If the suspended ventricle is pulsating, stimulation of the visceral nerve with a weak interrupted current causes inhibition of the rhythm in diastole, usually without any change in tonus. A typical tracing illustrating these inhibitory effects is reproduced in Fig. 10. The weakest stimulus to the nerve produces only a slight decrease in the strength of the beats. A greater strength of the interrupted current produces complete arrest of the rhythm together with a tonus relaxation greater than that during the normal diastolic pause. When the visceral nerves are being stimulated with an interrupted current

of slightly greater strength than suffices for complete inhibition, the paradoxical results shown at *b* and *c* (Fig. 10) are sometimes obtained. In each case there appears a marked tonus relaxation, but the effects on the rhythm can hardly be called either inhibitory or accelerating, as the rate of the beats is reduced but the strength of the beats augmented, and the accelerator effects are further shown by the peculiar grouping of the beats, which in some cases approach an incomplete tetanus. Records like these were frequently obtained, but the augmentation in the strength of the beats was not always so conspicuous as in this tracing. Preparations of the systemic ventricle and the visceral nerve of Loligo that show a regular rhythm after the ventricles have been isolated and subjected to the experimental conditions are the exceptions. The ventricular rhythm usually exhibits many irregularities, a frequent one being a definite grouping

FIGURE 10. — Tracing from the systemic ventricle of Loligo on stimulation of one of the visceral nerves. Showing inhibition of the rhythm (*a*, *b*), and a peculiar grouping of the "escape" beats bordering on incomplete tetanus when an interrupted current is used of slightly greater strength than suffices to produce complete inhibition (*c*).

of the beats in series of two or three. In preparations showing this type of rhythm the stimulation of the nerve produced in most cases the usual inhibition in diastole; but with a minimal intensity of the stimulus the only effect produced in many preparations was a change from the irregular to a regular rhythm, that is, *the stimulation abolished the grouping of the beats*. On cessation of the stimulation the grouping usually reappeared.

In four preparations I obtained what appeared to be actual acceleration of the ventricular rhythm by stimulation of the visceral nerves. Three of these were with the heart *in situ*, but the augmentation in the rate of the beats was so marked that it could not be mistaken. In one preparation the accelerator effects were obtained in the suspended ventricle. The ventricle beats in groups of two, and the strength of the interrupted current that first affects the heart increased the number of beats in the group, contrary to the usual effect. Increasing the strength of the stimulus slightly produced distinct acceleration of the beats, followed by a prolonged diastolic pause on cessation of the stimulation.

The auricles of the squid are almost too delicate for the graphic registration. They can, however, be separated more or less completely from the adjacent tissue, and severed from the gills so that changes in internal and external pressure are excluded; but when the auricles are thus prepared, rhythmical pulsations invariably cease. Stimulation of the visceral nerve has the same effect on the auricles in this condition as on the resting ventricle, namely, a series of beats on cessation of the stimulation, and in some cases also a beat at the beginning of the stimulation.

These results on the systemic heart of Loligo confirm the observations of Bert on Sepia. The systemic ventricle and the auricles are supplied with inhibitory fibres from the visceral nerves. The results indicate, furthermore, that the visceral nerves probably also contain accelerator fibres to the ventricle. The evidence to this effect is not conclusive, as definite augmentor effects were obtained in only four out of the great number of preparations examined. But the "paradoxical" response of the ventricle on stimulation of the visceral nerve (Fig. 10), which was of more frequent occurrence, may indicate the presence of cardio-accelerator nerves. Ransom has obtained similar reactions in the systemic ventricle of Octopus on stimulation of the visceral nerve with a strong interrupted current. According to Ransom's view the stimulation of the inhibitory fibres in the visceral nerve produces anabolism in the ventricular muscle, which for a time renders katabolism, or beats, impossible; but when this anabolic process has reached a certain maximum, the opposite process sets in and the ventricle "escapes" from the inhibitory influence, the resulting beats being of greater strength than the normal in virtue of the material built up through this "trophic" influence of the nerve. Ransom applies the same explanation to the augmentation of the rhythm that sometimes follows the cessation of the stimulation of the visceral or inhibitory nerve. On this theory of the nature of the action of the inhibitory nerves (on the muscle) it is difficult to understand how inhibition of the beats can ever be produced by stimulation of the nerves without a subsequent augmentation of the rate and the strength of the beats on cessation of the stimulation. It is true that such augmentation is usually present, but not invariably so. In Mya, Tapes, Venus, Triopha, Ariolimax, Limax, Helix, Loligo, Octopus, and Palinurus, the cardiac rhythm may be completely arrested in diastole by stimulation of the inhibitory nerves, and the rhythm that follows the cessation of the stimulation

is the same as that preceding the stimulation. The inhibition with subsequent augmentation of the rhythm is just as readily explained on the assumption that the nervous impulses prevent those processes which result in contraction, without affecting the anabolic processes; as under such conditions the anabolism would gain on the katabolism during the cessation of the beats, and provided the rate and the strength of the beats stand in direct relation to the end products of anabolism, cessation of the stimulation or inhibition would of necessity be followed by an improved rhythm.

There is nothing known in the physiology of muscle or nervous tissue which would indicate that the processes of anabolism tend to check or inhibit the counter processes of katabolism. On Ransom's theory the rapid succession of beats that follow each supermaximal beat during the stimulation of the nerve in Fig. 10, *b*, *c*, is simultaneous with the absence of anabolic processes or inhibition. If that was the case, one would expect to see similar extra beats or incomplete tetanus on cessation of the stimulation, but when the stimulation of the nerve ceases the ventricular rhythm assumes quite a different form. This goes to show that the peculiarity of the rhythm during the stimulation cannot be accounted for solely by the absence of inhibitory influences. But further work is required to decide whether these paradoxical reactions of the ventricle on stimulation of the visceral nerve are caused in whole or in part by simultaneous stimulation of two antagonistic nervous mechanisms.

The gill ventricles are still more difficult to isolate so that contraction of adjacent musculatures does not affect them. The ventricles are partly invested by a delicate muscular envelope of the same color as the ventricles themselves. The visceral nerves contain motor fibres to this musculature, and stimulation of the nerves tetanizes the muscle and compresses the ventricles. The muscular and connective tissue envelopes are not easily removed without injury to the ventricles and severance of the nervous connections. When the ventricles are severed from the gills and the renal veins, and separated as much as possible from the investing tissues, the effect on the rhythm is the same as on the systemic heart, that is, inhibition of the beats in diastole accompanied by a tonus relaxation. The tonus relaxation and consequent dilation of the ventricles is so marked that there is no possibility of construing the cessation of the rhythm as a tetanus or state of extreme contraction. The inhibition of the gill ventricles on stimulation of the visceral nerves may be

maintained for a longer time than the similar inhibition of the systemic ventricle; and cessation of the stimulation is usually followed by an augmented rhythm.

In rare instances the stimulation of the nerves caused irregularities in the rhythm without any distinct inhibition; and in three or four preparations the stimulation appeared to cause actual acceleration of the rhythm similar to that already described in the systemic ventricle. If the gill ventricles are quiescent, they usually give one or two beats at the beginning of the stimulation and then remain in diastole till the stimulation of the nerves ceases, when a more or less prolonged series of beats follow. It is therefore evident that *the influence of the visceral nerves is the same both on the systemic and the gill ventricles; the nerves send inhibitory fibres, and possibly also accelerator fibres, to all three ventricles.*

The effects on the gill ventricles of stimulation of the visceral nerves are duplicated by stimulation of the nerves in the gills. When the visceral nerve is severed from its central connections, and the gill ventricle separated from the gill and the renal veins, leaving the nervous connections to the ventricle intact, stimulation of the isolated nerve in the gill with a weak interrupted current produces invariably inhibition in diastole of the gill ventricle at the beginning and during the first part of the stimulation. The rhythm usually reappears after a few seconds' inhibition, whether the stimulation is continued or not; and I never succeeded in obtaining complete inhibition for the length of time possible by stimulation of the visceral nerve. The cessation of the stimulation is usually marked by an augmented rhythm. In no instance did the stimulation produce acceleration of the rhythm. The effect on the ventricle could not have been produced by the escape of the current on to the ventricle itself, for when the gill nerve is severed between the electrodes and the ventricle, and the ends of the nerve placed so as to overlap, stimulation of the nerve peripheral to the lesion has no further effect on the ventricle. We have, therefore, to do with an inhibitory reflex from the gill through the ganglion at the base of the gill (or the ganglion on the gill ventricle itself) to the gill ventricle.

The renal veins are supplied with nerve fibres from the visceral nerves; but it is wellnigh impossible to isolate the veins so that they are not affected by the movements of the cardiac apparatus and the viscero-pericardial sac and at the same time leave their nervous supply intact, and for that reason it is difficult to determine the function

of the renal nerves. There are indications that the visceral nerves send inhibitory fibres to the rhythmically active parts of the renal veins, just as to the rest of the cardiac apparatus. The question will be referred to again in connection with the same subject in Octopus.

These experiments on the smaller squid of the Pacific Ocean were repeated on the larger representative which is obtained in abundance at Woods Holl, with substantially the same results. This larger animal makes the work easier and the results more reliable. I found that after lesion of the visceral nerves between the visceral commissure and the point of origin of the nerves to the auricles and the gill ventricles stimulation of the visceral nerves central to the commissure still produced the characteristic effects on the systemic ventricle. This proves that nerve fibres pass to the ventricle from the visceral nerves at the level of the commissure, as I have shown to be the case in Ommastrephes. If the visceral nerves are stimulated peripheral to the commissure, the usual effects on the auricles and the gill ventricles are produced, but the systemic ventricle is not affected. It would thus seem that the condition found in so many of the gasteropods also obtains in Loligo, namely, that *the auricular nervous supply does not extend to the ventricle and the ventricular nerves do not influence the auricles.*

Owing to scarcity of material, I have been able to make only a few observations on the physiology of the heart and the neart nerves of Ommastrephes, but so far as these go there appears to be only this difference between this larger species and Loligo. Ommastrephes is much more tenacious of life, its muscle and nerve less excitable, the rate of the heart-beat only about two-thirds that of Loligo, and the cardiac rhythm is maintained with perfect co-ordination for from twenty to thirty minutes after the mantle has been slit open, a stream of water being passed over the gills. This operation immediately destroyed the co-ordination of the heart in Loligo.

The behavior of the various parts of the cardiac system of Octopus on stimulation of the visceral nerves has been described at some length by Ransom and by Bottazzi and Enriques. The opening of the mantle cavity is much less fatal to the co-ordination and rhythm of the heart in Octopus than in Loligo. When the mantle of Octopus is slit open in the ventral median line, the animal fixed on its back and the flaps of the mantle pinned to either side so as to expose the heart, the rhythmical pulsations and normal sequence of the beats in the different parts (renal veins, gill ventricles, auricles, systemic ven-

tricle) continue for a few minutes, even if the preparation is exposed to the air; but when the preparation is kept covered with sea-water a regular rhythm and synchrony of beats are maintained for one-half to three-fourths of an hour after the operation.

Before going into the detailed description of my results, it may be stated that they agree perfectly with those of previous observers as regards the function of the nerves to the auricles and the systemic ventricle, but are directly contradictory as regards the nerves to the branchial ventricles; in other words, *stimulation of the visceral nerves causes inhibition in diastole of the isolated gill ventricles as well as of the auricles and the systemic ventricle.*

Ransom studied the action of the visceral nerves on the filled systemic ventricle mainly. His published tracings are very similar to those obtained by me from the suspended empty ventricle. The resting ventricle usually responds with one or two beats at the beginning of the stimulation of the nerves, and the cessation of the stimulation is marked by a series of beats. The pulsating ventricle remains in complete or partial diastole during the stimulation, and on its cessation the beats reappear, sometimes with increased strength and rapidity. The arrest of the ventricle in diastole is invariably accompanied by a tonus relaxation, if through injury or stimulation the tonus of the muscle is rather greater than normal; otherwise there is no change in the tonus.

When the visceral nerves are stimulated, the viscera and the cardiac apparatus remaining intact, the gill ventricles are pressed against the viscera and appear somewhat contracted. They either remain quiescent in this condition during the stimulation or irregular beats occur, the rate of pulsation being usually less and only in exceptional cases greater than in the original rhythm. The cessation of the stimulation is marked by a greatly augmented rhythm. Ransom describes the condition of the ventricles during the stimulation of the nerves as a state of "extreme contraction." Even direct observation will suffice to show that this is incorrect, even in reference to the ventricles in which the arrest of the beats is complete. The contraction has all the appearance of a passive compression or displacement due to contraction of the surrounding muscular tissue. The gill ventricles are closely invested by a muscular membrane, which is of a different color from the ventricular muscle and can therefore be readily distinguished, but it adheres so closely to the surface of the ventricles that its complete removal in the region of the reno-ven-

tricular opening and at the place of entrance of the nerves is practically impossible without great injury to the ventricles; and slight injury to the ventricles is unavoidable, even when only partial isolation is attempted. When the ventricles are thus isolated stimulation of the visceral nerves with a weak interrupted current invariably produces inhibition in diastole of the pulsating ventricle and does not affect the quiescent ventricle, the cessation of the stimulation being followed in the former case by the original or a slightly augmented rhythm, in the latter preparation by a series of beats.

The isolated gill ventricles lend themselves to the graphic method almost as readily as the systemic ventricle. For this purpose the ventricles were freed as much as possible from the investing tissues, and suspended by means of a silk ligature at the junction of the afferent gill sinus and a platinum hook in the posterior end of the ventricle. The reno-ventricular and the ventriculo-branchial openings are not exactly at the opposite ends of the ventricle, so that if it is suspended by the branchial sinus and the renal vein, the lever does not record the full magnitude of the beats. When the isolation of the ventricle from the investing musculature is only partially successful, stimulation of the visceral nerve with the interrupted current produces what appears at first to be a tonus contraction in the ventricle. This contraction is undoubtedly what previous investigators have taken to be tetanus or extreme contraction of the ventricle itself, and in consequence ascribed accelerator function to the nerve. If no pains are taken to isolate the ventricle from the muscular envelope, the persistent contraction is much more marked. But that these persistent contractions are neither tetanus nor tonus contractions of the heart muscle, but due to contraction of the muscular tissue that still adheres to the surface of the ventricle, is shown by the fact that the more complete the isolation of the ventricle the less evident the contraction.

The typical inhibitory effects on the gill ventricle of stimulation of the visceral nerve are illustrated in Fig. 11. At *a* the strength of the interrupted current is relatively weak, and the result is incomplete inhibition, the rate of the beats remaining unchanged but the strength of the beats being greatly reduced. In other cases the decrease in the strength is also accompanied by decrease in the rate of contraction. At *b* the intensity of the stimulus is increased with the result that the inhibition of the rhythm in diastole is complete. There is no apparent latent period between the beginning of the stimulation

of the nerve and the appearance of the inhibitory effects on the ventricle. The suspended gill ventricles showed not infrequently a very irregular rhythm, probably due to unavoidable injuries in the preparation, but the influence of the visceral nerves is the same as in the preparations that exhibited no such irregularities.

In five preparations the stimulation of the visceral nerves produced diminutive beats in the gill ventricle, but the rate of the beats was increased. In no case did the stimulation of the nerve produce augmentation of the strength of the beats. It has already been stated that with the whole cardiac apparatus intact stimulation of the visceral nerves produces in exceptional cases actual acceleration of the rhythm in the gill ventricles; but the displacement of, and abnormal pressure on the gill ventricles under those conditions are

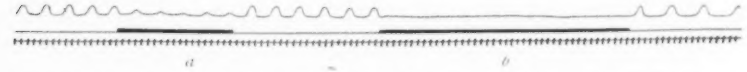

FIGURE 11.—Tracing from the gill ventricle of Octopus on stimulation of the visceral nerve, showing inhibition.

so considerable that the acceleration may be caused by these stimuli. This cannot be the case in the isolated and suspended preparation; nor do I think that the effects are produced by the escape of the current directly on to the ventricle, because when these reactions were obtained the strength of the interrupted current employed was not greater than usual and the electrodes were placed on the isolated nerve at least 5 cm. from the ventricle. It seems therefore probable that the visceral nerves send both augmentor and inhibitory fibres to the gill ventricles, the influence of the latter being by far the greatest.

A single induced shock applied to the visceral nerve is without effect on the gill ventricle unless of an intensity that surely injures the nerve. When of moderate strength the induced shocks must be applied to the nerve at the rate of one or two per second, in which case the effect on the ventricle is the same as when the interrupted current is used, that is, partial or complete inhibition in diastole, the greater the rate of application of the shocks the more complete the inhibition.

When the inhibition of the rhythm in the systemic ventricle following the stimulation of the visceral nerves is accompanied by any change in the tonus, it is invariably a relaxation. The same is true of the systemic ventricle and the gill ventricles of Loligo.

A similar action of the inhibitory nerves can be demonstrated also for the gill ventricles of Octopus under certain conditions. When the ventricles are stimulated directly with a strong interrupted current a strong persistent contraction is produced which may last for many seconds after the stimulation has ceased; and when the visceral nerve is stimulated with the ventricle in this state of persistent contraction a prompt relaxation is produced, lasting as long as the stimulation of the nerve. The suspended ventricle was made a part of the secondary circuit in the manner described for the ventricle of Aplysia,[1] and by means of a Pohl's commutator without

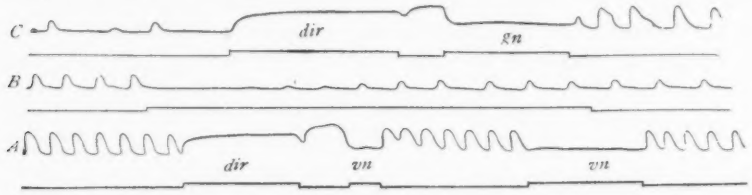

FIGURE 12. — Tracings from the gill ventricle of Octopus. *A*, *dir* = direct stimulation of the ventricle with a strong interrupted current. *vn* = stimulation of the visceral nerve, showing relaxation of the "tonus" contraction. *B*, reflex inhibition of the gill ventricle on stimulation of the gill nerve. *C*, *dir* = direct stimulation of the ventricle with the strong interrupted current. *gn* = stimulation of the gill nerve, showing "tonus" relaxation.

cross bars the induced current was sent through the heart or through the stimulating electrodes at will. In Fig. 12 *A*, a tracing illustrating these reactions is reproduced. At the left-hand side the strong interrupted current is sent directly through the ventricle with the results just described. On cessation of the stimulation the height of contraction is even increased. Sending the weak interrupted current through the visceral nerve (*vn*) produces immediate relaxation, but on repeating the stimulation of the nerve the inhibition in diastole is effected without any change in the tonus. It is therefore evident that the influence of the visceral nerve on the tonus of the gill ventricles is the same as that on the systemic ventricle; if the stimulation of the nerve affects the tonus, it is in the line of relaxation.

The effects on the gill ventricles of stimulation of the visceral nerves are almost duplicated by stimulation of the nerves in the gills, just as has been described in Loligo; but in Octopus the gill ventricles

[1] CARLSON: This journal, 1905, xiii, p. 419.

are strong enough for graphic registration, so that the nature of this reflex can be studied to better advantage. For this series of experiments I usually isolated both the visceral nerve and the gill nerve, and the nerves were placed on separate pairs of electrodes, connected with a Pohl's commutator, so that either nerve could be stimulated at will; the suspended ventricles were at the same time made to complete another secondary circuit, so that the influence of the gill nerve, of the visceral nerve, and of direct stimulation of the ventricle itself could be studied in the same preparation. A comparison of Fig. 12, *B*, with Fig. 11 shows plainly the close parallel of the influence of the visceral nerve and the gill nerve on the rhythm and tonus of the gill ventricle. The reflex from the gill nerve is manifested in inhibition of the rhythm (Fig. 12, *B*) and decrease of the tonus (*C*); but these effects are, as a rule, not as marked nor as long continued when brought about by stimulation of the gill nerve as when caused by stimulation of the visceral nerve.

Are the systemic ventricle and the auricles of the cephalopods provided with accelerator nerves? Fredericq concluded on physiological grounds that accelerator nerve fibres to the systemic ventricle of Octopus passed alongside or in the walls of the cephalic vena cava; but Ransom has since shown that Fredericq's method of observation was at fault. Accelerator nerves to the systemic ventricle of Octopus have more recently been described by Bottazzi and Enriques. These investigators state that the stimulation of the gastric ganglion, the œsophagus, the cephalic artery, or the small arteries that pass to the œsophagus from the cephalic artery, produces acceleration of the rhythm in the systemic ventricle. The accelerator nerves, they conclude, pass from the gastric commissures in the œsophagus along these small arteries to the cephalic artery and along it to the ventricle.

Before I was acquainted with the work of Bottazzi and Enriques I had tried in Loligo, but failed to find any effect on the systemic ventricle of stimulation of the cephalic artery and of the stomatogastric commissures in the œsophagus; and having read their paper I repeated the experiments with the same negative results. The gastric ganglion cannot very well be exposed and stimulated with a view of ascertaining its influence on the systemic ventricle, because the dissection destroys the connections between the dorsal side of the ventricle and the viscera, so that negative results do not mean much, as the nervous connections might be severed by the dissection. The

cephalic artery and the cephalic end of the œsophagus can be isolated for stimulation, leaving the cardiac apparatus intact. The stomato-gastric commissures pass to the gastric ganglion in the walls of the œsophagus, and stimulation of the œsophagus involves in consequence the stimulation of these commissures. Because of lack of material I have not repeated the experiments on Octopus; but the physiology of the cardiac apparatus is so nearly alike in the two groups of dibranchiate cephalopods, that if such an accelerator nervous mechanism exists in Octopus it would probably not be absent in Loligo. It seems to me, therefore, that the evidence for the existence of accelerator nerves to the systemic heart outside the visceral nerves is not conclusive.

Ransom concludes that the visceral nerves have only an accelerator influence on the renal veins in Octopus, while Bottazzi and Enriques are unable to affirm anything as to the innervation of these structures. A nerve enters the vena cava at its cephalic end in Loligo and Ommastrephes, but it cannot be isolated in the living specimens for the purpose of these experiments, and the function of this nerve was therefore not determined. Branches from the visceral nerves enter the renal veins in Loligo, and Ransom affirms the same for Octopus. But the study of the function of these nerves meets with the same difficulties that we encountered in the gill ventricles, namely, the difficulty of their complete isolation from adjacent tissues while retaining the nervous connections intact. This is particularly true in Loligo, in which the renal veins are exceedingly delicate. In Octopus the kidney allows a more complete isolation. When the renal veins in Octopus are laid bare by removing the visceral envelope, and the visceral nerves stimulated with a weak interrupted current, the whole kidney mass contracts; but if one observes the rhythmically contracting glandular appendages it appears that the contraction is of the same kind as that of the intact gill ventricles following stimulation of the visceral nerves, that is, the diminution in size of the kidney is not produced by the contraction of the rythmically pulsating parts, but by the contraction of other muscular tissue around and possibly in the walls of the renal sinuses. So far as the pulsating renal sacs are concerned the contraction has all the appearance of being a passive one, and at no time can it be called "extreme." Careful observation will show that when pulsations occur during the contraction produced by stimulation of the visceral nerve, no previous relaxation is noticeable, and that during the systole of

these beats the size of the renal sacs is further reduced. The behavior of the renal veins during the stimulation of the visceral nerves can best be explained on the assumption that the nerves contain inhibitory fibres to these organs. A further indication of inhibitory nerves to the renal veins is the inhibition of the cardiac and the renal movements that is sometimes observed when the mantle is first slit open. All the cardiac organs as well as the renal veins remain quiescent in extreme diastole; but as soon as the visceral nerves are severed, the cardiac rhythm and the pulsations of the renal veins reappear. This arrest of the cardiac rhythm is evidently a reflex inhibition caused by the injury to the mantle. The behavior of the renal veins is exactly the same as that of the cardiac organs which are supplied with inhibitory fibres from the visceral nerves.

III. Summary.

1. With the exception of Mytilus, in which the results are not conclusive, the auricles and the ventricle of the lamellibranchs are supplied with inhibitory nerves from the visceral ganglion or ganglia. These nerves enter the heart at the base of the auricles.

The heart of the chitons is in all probability supplied with augmentor nerves from the pleuro-visceral cords. The heart of the prosobranchs (Haliotis, Lucapina, Natica, Sycotypus) and tectibranchs (Aplysia, Bulla, Pleurobranchæa) is supplied with augmentor nerves from the visceral ganglion or ganglia. These nerves enter the ventricle at the aortic end. The auricular nerves enter the auricle at its base.

The heart of the nudibranch Montereina is similarly provided with augmentor nerves. The heart of Triopha, another nudibranch, appears to be provided with both inhibitory and augmentor fibres.

The auricle of the slugs (Ariolimax, Limax) and the snail (Helix) is supplied with both inhibitory and augmentor nerves from the subœsophageal or pleural ganglion. The nerve-fibres enter the auricle at its base. The innervation of the ventricle in these pulmonates is less uniform. The ventricle of Helix is supplied with both augmentor and inhibitory fibres which enter the ventricle at the aortic end; some fibres also reach the ventricle through the walls of the auricle. The ventricle of Limax appears to be supplied with only inhibitory nerves which reach the ventricle through the auricular walls. The ventricle of Ariolimax is supplied with augmentor, and probably also

inhibitory, nerves, which enter at the aortic end. In Helix the influence of the inhibitory, in Ariolimax that of the augmentor, fibres is the greater.

The systemic and the branchial heart of the cephalopods are supplied with inhibitory nerves from the two visceral commissures, and there is some evidence that augmentor fibres reach the hearts from the same source. The rhythmically contracting parts of the renal veins are in all probability also supplied with inhibitory fibres from the visceral commissures.

2. The inhibitory effects on the heart of stimulation of the inhibitory nerves appear in complete arrest of the heart in diastole or in diminished rate and amplitude of the beats. The inhibition is usually accompanied by tonus relaxation. The physiology of the cardio-inhibitory nerves to the molluscan heart is thus identical with that of the cardio-inhibitory nerves in the vertebrates.

In the quiescent heart the stimulation of the augmentor nerves produces contraction or a series of contractions.[1] The augmentor fibres thus partake of the character of true motor nerves.

Single induced shocks applied to the cardiac nerves are usually ineffective, unless of considerable intensity. The cardiac nerves are stimulated during the passage of the constant current through them.

3. Inasmuch as this work includes representatives from all the different groups of the phylum, with the exception of the pteropods, it may be inferred that the molluscan heart is without exception provided with cardio-regulative nerves, augmentor or inhibitory or both. A further general conclusion is this, that in the lowest molluscs (chitons, prosobranchs, tectibranchs) there seem to be only augmentor nerves present; gasteropods standing higher in the scale have both augmentor and inhibitory nerves; while the highest molluscs (the cephalopods) have only inhibitory heart nerves, or the inhibitory nerves are at least greatly in predominance. It is possible, of course, that both kinds of cardiac nerves are present throughout the phylum, and that in the lowest group the augmentor, in the highest the inhibitory, fibres predominate to the extent that on simultaneous stimulation of both kinds of fibres the influence of those in the minority never comes to light. Further work must be done on the heart-nerves in the nudibranchs, in which the inhibitory nerves make their first appearance.

[1] CARLSON · This journal, 1904, xii, p. 55.

This peculiar distribution of the augmentor and the inhibitory nervous mechanisms in the molluscs suggested the idea that the augmentor mechanism may antedate the inhibitory in the time of development. So far as I know the literature contains no data touching this question, save what may be inferred from the action of drugs on the embryonic heart. This question touches the development of inhibitory processes in general inasmuch as cardiac inhibition is in all probability only a special case of inhibitory processes in or between nerve-centres.[1] One of the difficulties met with in working out the facts in the case is the low excitability of the vagi in early embryonic life. In dog embryos two centimetres in length, I find that the stimulation of the vagi inhibits the heart, but the strength of the interrupted current required to produce this effect is so considerable that escape of the current directly to the heart can probably not be prevented. And observations on the invertebrate heart make it highly probable that a certain strength of the induced current applied directly to the heart produces inhibition even in the absence of an inhibitory nervous mechanism or after the inhibitory nervous mechanism has been paralyzed by drugs.

[1] CARLSON: This journal, 1905, xii, p. 217.

PROTEIN METABOLISM IN CYSTINURIA.

By CARL ALSBERG and OTTO FOLIN.

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IN a recent paper on cystinuria Loewi and Neuberg[1] cite experimental data and draw conclusions the importance of which must have appealed to all readers interested in the chemistry and metabolism of protein.

Loewi's and Neuberg's presentation of the subject may be briefly summarized as follows:

The cystin obtained from urine in cystinuria and the cystin which K. A. H. Mörner has taught us how to obtain directly from protein bodies have the same chemical constitution, *i. e.*, both are derived from Friedman's α-amido-β-thio propionic acid, $CH_2SH - CH\ NH_2\ COOH$ (cystein). The cystin obtained from so-called cystin stones, on the other hand, has a different constitution, according to Neuberg, being the corresponding derivative of α-thio-β-amido propionic acid, $CH_2 . NH_2 - CH . SH\ COOH$.

Both kinds of cystin are present in protein, and both kinds, whether given in the form of food or in free crystalline condition, are nearly quantitatively converted into sulphates in the metabolism of normal persons. The cystinuric individual, on the other hand, katabolizes normally only one kind of cystin, namely, that which occurs in cystin stones. The other, ordinary cystin obtained from urine or directly from protein, is not similarly hydrolyzed and its sulphur oxidized into sulphates. It passes unchanged through the system and is quantitatively eliminated with the urine.

The inability of the cystinuric individual to hydrolyze and oxidize the one kind of cystin does not represent all that is abnormal about his metabolism. Neuberg and Loewi conceived the idea that in cystinuria there is a much more general metabolism disorder, a more or less complete inability to normally katabolize amido acids. In their

[1] Loewi and Neuberg: Zeitschrift für physiologische Chemie, 1904, xliii, p. 338.

attempts to verify this idea Neuberg and Loewi first analyzed the urine of their patient with the expectation of finding that it contained amido acids in unusual amounts; but except for the presence of the cystin they found the urine to be normal. On feeding pure amido acids to their patient they did, however, discover the metabolism abnormality which they were looking for. In the normal person such amido acids are quantitatively katabolized, and their nitrogen eliminated as urea. Their patient did not convert the nitrogen of amido acids into urea, but eliminated the monoamido acids unchanged and almost quantitatively, while the diamido acids were eliminated as the corresponding diamines.

This remarkable condition is demonstrated with a rigidity that seems to leave no room for doubt or question. In each case nearly all the product fed was recovered in the urine, and its identity and purity established by analyses. Thus after feeding 6.02 gm. of tyrosin 4.82 gm. chemically pure tyrosin were recovered from the urine; 5 gm. of asparaginic acid yielded 3.37 gm. of the same substance in the urine; and when 6 gm. of protein cystin were fed 5.8 gm. were recovered.

Since the cystinuria patient is unable to utilize normally pure acids but can and does utilize the amido acid contents of ordinary food, Neuberg and Loewi further advance the hypothesis that in the digestive tract food protein is normally not split to any great extent into amido acids, as such food protein should otherwise be eliminated not as urea but as amido acids in the case of cystinuric individuals.

The more we have studied the experimental data presented in the paper of Neuberg and Loewi the less reason have we had to question the accuracy of the work except in one or two minor particulars. Our own experiments to be described in this paper have, however, entirely failed to corroborate their findings.

Our investigation was not undertaken merely for the purpose of verifying the results of Loewi and Neuberg. One of us (A.) had been engaged in the study of a case of cystinuria for some time before the appearance of their paper, and a partial report of the results obtained was published last December.[1] We planned to make a general metabolism study of a case of cystinuria using the standard uniform diets recently described in this journal.[2]

By comparison with the numerous data already recorded in connection with the use of those diets we hoped to show more definitely than

[1] ALSBERG: Journal of medical research, 1904, xiii, p. 105.

[2] FOLIN: This journal, 1905, xiii, pp. 64, 73.

had been done the degree and nature of the deviations from the normal metabolism associated with cystinuria. In view of the results recorded by Loewi and Neuberg it seemed not improbable that the composition of the cystinuria urine corresponding to the standard protein-rich diet should after all be characterized by the presence of notable quantities of amido acids, because we could not accept their hypothesis that such amido acids are not produced within the digestive tract. If there were a general disturbance in the amido acid katabolism of the case under investigation, it was to be expected that the undetermined nitrogenous residue (the nitrogen not present in urea, ammonia, uric acid, and kreatinin) should be greater than in normal persons, and that such a deviation from the normal should disappear with the introduction of a diet containing little or no protein. These expectations were based on the assumption that the general results obtained by Neuberg and Loewi with amido acids were correct, but they had not analyzed the ordinary urine of their patient with sufficient care.[1]

In connection with our metabolism experiments, especially with a low nitrogen diet, we intended to repeat and to extend the studies of Loewi and Neuberg in regard to the fate of pure amido acids when administered to a cystinuric patient. Our scheme of analysis would readily have enabled us to verify their findings with regard to any amido acid which is not decomposed at 160° (the temperature of the urea determination). This plan was, however, abandoned after we had satisfied ourselves that the metabolism in our case of cystinuria did not at all tally with the description given by Neuberg and Loewi.

The patient was a young man twenty-three years of age. He was first known to "pass sand" at eleven (in 1893) and had considerable trouble of the same sort during the years 1897–1899. In 1903 he was admitted to the Massachusetts General Hospital, and several cystin stones were removed from his bladder. One of his brothers has been operated upon five times for bladder stones, but as far as he knew no other relative of his has had the same trouble.

The general plan of the feeding experiments recorded below is the same as that which prevailed in the earlier experiments already described in this journal.[2] The analytical technique employed is the

[1] They have not stated how they determined the urea, and have made no reference to the ammonia, thus leaving it uncertain whether the urea-nitrogen did or did not include the ammonia-nitrogen.

[2] *Loc. cit.*

same, except with regard to the sulphate determinations. The presence of cystin necessitated a different procedure for the determination of the total sulphur, sodium-peroxide being used as the oxidizing reagent.[1]

In Table I are recorded the last five days' results of a feeding experiment with the standard milk and egg diet, containing 119 gm. of protein, 148 gm. fat, and 225 gm. carbohydrates. At the bottom of the table are given the corresponding average maximum and minimum values previously recorded for the same diet when fed to normal persons (six).

Several unmistakable deviations from the normal are at once to be noted.

The neutral sulphur, including the cystin-sulphur, is, as was to be expected, very much higher than the normal, being in fact, almost five times as great in the case of the cystinuric patient. If this difference is due to cystin alone, as we believe to be the case (the reasons for this belief will be given below), then we find that our patient eliminated on the average on this diet $0.82 - 0.17 = 0.65$ gm. SO_3 per day in the form of cystin. On this diet our patient eliminated therefore almost one gramme (0.97 gm.) of cystin per day. Loewi and Neuberg determined directly by sedimentation the cystin obtained from their patient, and found only about one-half gramme per day. Our patient might, therefore, at first sight appear to present a more pronounced case of cystinuria than theirs, but this is not necessarily the case. Notwithstanding the contrary statements to be found in the literature, the daily amount of cystin eliminated depends to a notable extent upon the total amount of sulphur katabolized. Neuberg and Loewi paid no particular attention to the total sulphur katabolism of their patient, and their figures are therefore not strictly comparable with those here recorded.

The abnormally high neutral sulphur values given in cystinuria are produced wholly at the expense of the inorganic sulphates, as will be seen from the other sulphur columns. The ethereal sulphates are within the figures given by the normal persons.

Turning to the second half of Table I, it will be noted that the nitrogen metabolism of our patient also is markedly different from that

[1] It is believed that the sulphate values recorded in these experiments possess a higher degree of accuracy than it is possible to obtain according to directions given in text-books. But space cannot be taken here for a discussion of the methods used. The number of details involved will necessitate a separate paper on the subject.

TABLE I.

NITROGEN-RICH DIET.

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	Neutral SO_3. "S_3."	In per cent of Total Sulphur.			Titrated acidity. c.c. $\frac{N}{10}$.	Total phosphates as P_2O_5. gm.	Remarks.
					S_1.	S_2.	S_3.			
22	2.50	1.51	0.17	0.82	60.4	6.8	33.2	361	3.30	
23	2.80	1.75	0.21	0.84	62.5	7.5	30.0	412	3.75	
24	3.19	2.20	0.18	0.82	68.9	5.6	25.5	440	4.00	
25	3.11	2.18	0.21	0.82	70.1	6.7	23.2	423	3.88	
26	3.04	2.04	0.20	0.80	67.1	6.6	26.3	396	3.71	
Average	2.93	1.94	0.19	0.82	59.8	6.6	27.6	406	3.73	
Normal av.	3.31	2.92	0.22	0.17	87.8	6.8	5.1	617	3.87	See This journal, 1905, xiii, p. 63.
Normal max.	3.73	3.25	0.25	0.19	89.6	8.0	6.1	669	4.50	
Normal min.	3.11	2.67	0.19	0.13	84.7	5.5	4.1	554	3.44	

TABLE I — *Continued.*

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. gm.	Urea. gm.	Ammonia. c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. February.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined nitrogen.
59.2	*21*		*23.0*	*245.0*	*1.41*	*0.36*							
22	1530	13.26	10.7	0.34	0.52	0.12	1.7	80.8	2.5	83.3	3.9	0.9	12.8
	235		*23.0*	*192.0*	*1.35*	*0.32*							
23	1410	13.10	10.7	0.27	0.50	0.11	1.5	81.9	2.1	84.0	3.8	0.9	11.2
	235		*28.5*	*226.0*	*1.51*	*0.34*							
24	1450	15.59	13.3	0.32	0.56	0.11	1.3	85.3	2.0	87.3	3.6	0.7	8.4
	235		*29.1*	*180.0*	*1.33*	*0.36*							
25	1450	16.16	13.6	0.25	0.49	0.12	1.7	84.3	1.5	85.8	3.0	0.7	10.5
58.8	*24*		*29.1*	*201.0*	*1.35*	*0.36*							
26	1480	16.08	13.6	0.28	0.50	0.12	1.6	84.4	1.7	86.1	3.1	0.7	10.2
Average	1464	14.84	*26.5* 12.4	*209.0* 0.29	*1.39* 0.51	*0.36* 0.12	1.6	83.3	2.0	85.3	3.5	0.8	10.6
Normal average	1430	16.00	*29.8* 13.9	*500.0* 0.70	*1.55* 0.58	*0.37* 0.12	0.6	87.5	4.3	91.85	3.6	0.8	3.75
Normal maximum	1812	18.20	*34.7* 16.2	*608.0* 0.85	*1.77* 0.66	*0.46* 0.15	0.85	89.4	5.0	92.60	4.5	1.0	5.30
Normal minimum	1196	14.80	*27.3* 12.8	*392.0* 0.55	*1.36* 0.50	*0.24* 0.08	0.41	86.2	3.3	90.70	3.2	0.6	2.70

of the six normal persons. The per cent of the nitrogen represented by the urea is fully 4 per cent lower than the normal average, and is 3 per cent lower than the minimum value obtained from a normal person. Similarly with regard to the ammonia. The absolute quantity eliminated is scarcely more than one-half the minimum quantity obtained from any one of the six normal persons, and represents only 2 per cent of the total nitrogen as against an average of 4.3 per cent for the normal. The absolutely and relatively low ammonia elimination is, as a matter of fact, only what one would naturally expect from the abnormally large amount of sulphur eliminated in neutral form.

Accepting the excess of neutral sulphur (0.65 gm.) and calculating this into the equivalent quantity of $\frac{n}{10}$ ammonia (162 c.c.) would bring the ammonia elimination almost within the normal limits. But the free (titrated) acidity is also decidedly below the normal minimum. In addition to the deficiency in inorganic acids (sulphuric) it would seem, therefore, that either there must exist a deficiency in organic acids, or that some organic base is produced and in part takes the place of ammonia. The latter is perhaps the most probable explanation in view of the fact that tetra- and pentamethylene diamines have occasionally been found in cystinuria urines. One of us (A) made earlier a search for these diamines in the urine of this patient, but with negative results. Loewi and Neuberg also failed to find diamines in the urine of their patient, and according to them they are seldom present, having been found only three times. Cystinuria itself is, however, not a frequent occurrence, and it seems improbable that the diamines should have been found in such urines three times without their occurrence being a regular (more or less constant) part of the same metabolism disorder that gives rise to the cystin. It seems more likely that they are always or nearly always present in such cases, but that the amounts present have been too small for direct isolation and identification. From Simon's interesting discussion of cystinuria and diaminuria[1] it is clear that at least two additional cases of diaminuria associated with cystinuria must be added to the cases mentioned by Loewi and Neuberg.

The kreatinin and uric acid elimination is normal, and needs no discussion here. It will be taken up again in connection with Table III, p. 68.

Corresponding to the subnormal amounts of urea and ammonia eliminated by our patient on the standard nitrogen-rich diet, we find in Table I an unusually large amount of "undetermined" nitrogen,

[1] SIMON: American journal of medical sciences, 1900, xix, p. 48.

1.6 gm. as against an average of only 0.6 gm. for the normal persons, or 10.6 per cent of the total as against the normal 3.75 per cent.

The abnormal distribution of the eliminated nitrogen, recorded in Table I, seemed at first to verify our idea of the condition that exists in cystinuria. If the cystinuric individual is unable to make use of pure amido acids, it seemed to us probable that notable quantities of amido acids ought to occur in the urine when such an individual is taking food that is rich in nitrogen, and this amido acid-nitrogen would appear in our scheme of analysis as undetermined nitrogen. At the end of the experiment recorded in this table we were therefore still of the opinion that the generalizations of Loewi and Neuberg concerning the nature of cystinuria were substantially correct, but that they had not analyzed with sufficient care the urine as obtained from ordinary nitrogen-rich diets.

This interpretation was quickly shattered by the results which were obtained when the nitrogen-rich milk and egg diet was replaced by a practically nitrogen-free starch and fat diet (Table II).

If the abnormally high undetermined nitrogenous rest shown in Table I were due to the formation in the digestive tract of amido acids, which when absorbed would be eliminated in unchanged condition owing to the patient's inability to katabolize normally such amido compounds into urea, then it seemed reasonable to expect that with the substitution of a nitrogen-free diet the patient's metabolism should become more or less perfectly like that of a normal person. In other words, the undetermined nitrogen should sink to the level of that given by normal persons on the same diet. This does not happen. The published records of seven persons[1] on a similar diet show an undetermined nitrogenous rest of about 0.4 gm. In this case the undetermined nitrogen in the urine under the influence of a nitrogen-free diet remains practically stationary at about 0.8 gm.

But instead of dwelling on these deviations from the normal in the metabolism of our patient the reader's attention is at once called to the records in Table II of March 6th and 7th and March 9th and 10th respectively.

During the forenoon of March 9th the patient received in addition to the regular food exactly 10 gm. pure asparaginic acid (Kahlbaum's). The substance was given in small quantities of water in three doses about an hour and a half apart. The total nitrogen determinations

[1] *Loc. cit.*

TABLE II.

NITROGEN-FREE DIET.

Date. Feb.–March.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	Neutral SO_3. "S_3."	In per cent of Total Sulphur.			Titrated acidity. c.c. $\frac{n}{10}$.	Total phosphates as P_2O_5 gm.	Remarks.
					S_1.	S_2.	S_3.			
27	1.37	0.66	0.14	0.58	48.1	10.2	41.7	282	2.44	
28	0.99	0.31	0.09	0.59	31.3	9.1	59.6	210	1.77	
1	1.01	0.39	0.11	0.51	38.6	10.9	50.5	190	1.56	
2	0.97	0.36	0.12	0.49	37.1	12.4	50.5	160	1.11	
3	0.82	0.28	0.09	0.45	34.1	11.0	54.9	198	1.08	
4	0.77	0.20	0.07	0.49	26.0	9.1	64.9	185	0.82	
5	0.82	0.23	0.06	0.53	28.1	7.3	64.6	240	1.13	
6	1.16	0.61	0.07	0.46	52.6	6.0	41.4	199	0.74	1.226 gm. pure cystin (from hair) mixed with the diet.
7	0.99	0.43	0.09	0.47	43.4	9.1	47.3	253	1.11	
8	0.77	0.23	0.09	0.45	29.9	11.7	58.4	163	0.95	
9	0.81	0.24	0.10	0.47	29.6	12.3	58.1	149	0.89	
10	0.80	0.21	0.10	0.49	26.3	12.5	61.2	159	0.92	
11	0.80	0.23	0.09	0.46	29.9	11.3	58.8	127	0.94	

TABLE II — *Continued.*

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. gm.	Urea. gm.	Ammonia. c.c. $\frac{1}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. Feb.–Mar.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined nitrogen.
58.8	*14*	10.16	*16.8*	*270.0*	*1.26*	*0.28*	0.95	81.3	3.7	85.0	4.6	0.9	9.5
27	1500		8.27	0.38	0.47	0.09							
28	*12* 1500	6.63	*10.7* 4.99	*186.0* 0.26	*1.29* 0.48	*0.30* 0.10	0.80	75.2	3.9	79.1	7.2	1.5	11.6
1	*105* 2160	6.44	*9.5* 4.43	*181.0* 0.25	*1.34* 0.50	*0.14* 0.05	1.21	68.8	3.9	72.7	7.8	0.8	18.7
2	*95* 2100	5.42	*79.0* 3.72	*176.0* 0.25	*1.30* 0.52	*0.13* 0.04	0.89	68.6	4.5	73.1	9.6	0.7	16.6
3	*9* 1710	4.69	*6.2* 2.89	*225.0* 0.32	*1.35* 0.50	*0.16* 0.05	0.93	66.8	6.7	73.5	10.6	1.1	14.8
4	*11* 1290	4.33	*5.50* 2.57	*237.0* 0.33	*1.37* 0.51	*0.15* 0.05	0.87	58.8	7.9	66.7	11.6	1.2	20.5
5	*9* 1670	4.00	*4.80* 2.26	*320.0* 0.45	*1.33* 0.49	*0.11* 0.04	0.76	56.6	11.2	67.8	12.3	1.0	18.9
6	*10* 1605	3.65	*3.80* 1.79	*308.0* 0.41	*1.47* 0.55	*0.14* 0.05	0.85	49.7	11.2	60.9	14.9	1.4	22.8
7	*10* 1475	3.72	*3.70* 1.71	*348.0* 0.49	*1.33* 0.49	*0.12* 0.04	0.99	45.5	13.1	58.6	13.2	1.1	27.1
8	*8* 1940	4.11	*5.20* 2.42	*271.0* 0.38	*1.38* 0.51	*0.27* 0.09	0.71	56.9	9.3	66.2	12.2	2.2	19.4
9[1]	*65* 2330	4.47	*5.80* 2.71	*251.0* 0.35	*1.45* 0.54	*0.27* 0.09	0.78[1]	60.8	7.8	68.6	12.0	2.0	17.4
10	*8* 2090	5.00	*7.1* 3.29	*242.0* 0.34	*1.41* 0.52	*0.23* 0.08	0.77	65.7	6.8	72.5	10.4	1.6	15.5
58.5	*9*	4.89	*7.20*	*245.0*	*1.35*	*0.28*	0.62	68.3	7.0	75.3	10.2	1.8	12.7
11	2270		3.34	0.34	0.50	0.09							

[1] 10 gm. asparaginic acid given in the forenoon.

on the 9th, 10th, and 11th of March failed to show just how much of the asparaginic acid was absorbed, because the increase in the total nitrogen during those three days was greater than the asparaginic acid nitrogen could account for. But in 10 gm. asparaginic acid there is 1.05 gm. nitrogen. Any considerable absorption and subsequent elimination of unchanged asparaginic acid should therefore unquestionably have resulted in an unmistakable increase of the "undetermined" nitrogen of the urine. Not the slightest increase in this factor is to be observed. The increase in the total nitrogen is wholly due to an increase in the urea. This case of cystinuria is therefore quite well able to convert the nitrogen of asparaginic acid into urea.[1]

On March 6th 1.226 gm. pure cystin prepared from hair was mixed with the starch solution which together with butter (115 gm.) constituted the food during this period. On that day particular care was taken to have the food consumed quantitatively. If the generalizations of Neuberg and Loewi were correct, this cystin should have appeared unchanged in the urine during the following two or three days. 1.226 gm. of cystin is equivalent to 0.817 gm. SO_3. The increase in the total SO_3 elimination on March 6th and 7th is 1.16 + 0.99 − (0.77 + 0.82) or 0.56 gm. corresponding to 68.5 per cent of the cystin given. The increase in the inorganic SO_3 elimination on March 6th and 7th is 0.61 + 0.43 − (0.20 + 0.23) or 0.61 gm. The neutral sulphur elimination for the same two days is not at all increased. These figures prove beyond reasonable doubt that in our case of cystinuria pure cystin in so far as it was absorbed from the intestinal tract was not at all eliminated in unchanged condition, but that its sulphur gave rise to the normal katabolism product, sulphuric acid.

The results which we have obtained on feeding asparaginic acid and protein cystin to our patient make it clear that the disease known as cystinuria does not consist of a general inability to katabolize normally the hydrolytic cleavage product of protein. Whatever may be the explanation of the results reported by Loewi and Neuberg, their generalizations concerning the disease are not correct.

The neutral sulphur values given by our patient on the protein-rich and the protein-free diets correspond (after the normal neutral

[1] When heated with magnesium chloride and hydrochloric acid exactly as in the urea determinations 0.6646 gm. asparaginic acid gave only 1.8 c.c. $\frac{N}{10}$ NH_3 or 2.5 mgm. nitrogen.

sulphur has been subtracted) to about 1 gm. of cystin per day on the former and 0.5 gm. on the latter.

That these calculated cystin values are approximately correct is indicated by the following facts: The urines derived from the protein-rich diet contained even when perfectly fresh a perceptible sediment of typical cystin crystals, which increased on standing until a very voluminous sediment had formed. This sediment was found microscopically to consist to a large extent of cystin crystals. Under the influence of the protein-free diet this tendency of the urine to form a cystin sediment diminished rapidly, yet to the very end of the second feeding experiment (with starch and butter) a few cystin crystals could be found by the help of the centrifuge and the microscope after the urine had been allowed to stand a few days. Thus after standing about two weeks a few crystals were found adhering to the bottom of the bottle containing the urine of March 10th. The volume of urine passed on that date was 2090 c.c. This urine must therefore be considered as a slightly supersaturated, or at least as a saturated solution of cystin (or no crystals could have been formed). The total neutral sulphur for the day was 0.49 gm. expressed as SO_3. Subtracting from this the average normal figure 0.17 gm. SO_3, we get 0.32 gm. SO_3 or 0.48 gm. cystin.

There is certainly no reason for assuming that less than this amount of cystin was present, since it would correspond to a solubility of only 0.023 per cent or less than one-half the solubility ascribed to cystin in urine by Mester[1] and Borissow.[2] Nor can we assume that much more was present without also making the gratuitous assumption that there is no normal neutral sulphur in cystinuria urines.

At all events we can positively state that our patient continued to eliminate cystin with the urine at the end of a thirteen-day feeding experiment with a diet containing practically no protein at all. The analytical figures also prove that less neutral sulphur is eliminated on this diet than on the protein-rich diet. It would therefore seem almost, if not absolutely, certain that more cystin is produced and eliminated on a diet rich in protein than on one containing but little sulphur. The practical significance of this in the treatment of such patients is self-evident. They should abstain as much as is practicable from the use of highly nitrogenous foods.

It does not come within the scope of this paper to discuss normal metabolism in the light of the results obtained from an abnormal

[1] Mester: Zeitschrift für physiologische Chemie, 1889, xiv, p. 113.

[2] Borissow: Zeitschrift für physiologische Chemie, 1894, xix, p. 517.

case. The peculiar behavior of our patient on the one hand toward protein and on the other toward cystin, a cleavage product of protein, must, however, be considered. On the protein-rich diet our patient eliminated considerably more neutral sulphur than on the diet containing no protein; yet when cystin was fed together with the latter diet there was no increase in the neutral sulphur output. Why is this? One would naturally expect that at least an equally great fraction of the cystin-sulphur as of the protein-sulphur should escape oxidation and be eliminated in neutral condition. The actual state of affairs is, it will be noted, almost exactly the reverse of that reported by Neuberg and Loewi. Both their result and ours might fairly be questioned. Their statement that protein-cystin when administered in pure crystalline condition passed quantitatively through the system of their patient without change while the food sulphur was only in part eliminated as cystin, is manifestly one that will need further verification, and we admit readily that our result as stated above may also fairly be looked upon as needing verification. We feel positive of the accuracy of our result, but the same is undoubtedly true of Neuberg and Loewi.

It seemed possible that our giving such a small amount of cystin and in such small doses (it was mixed with the day's food) was the reason for our result, and that if a larger quantity in larger doses was given a part of the cystin might pass into the urine unchanged. To test this we made another feeding experiment, and this time gave practically the same quantity of cystin as Neuberg and Loewi had used (6 gm.), and all was given in the forenoon in three doses of 2 gm. each.[1]

The results of this experiment are recorded in Table III. The cystin was given on the fourth day, May 11th. The rise in the total sulphur elimination due to the cystin on May 11th and 12th is $2.03 + 1.91 - (0.96 + 0.97) = 2.01$ gm. SO_3. The corresponding rise in the inorganic sulphate elimination for the two days is $1.35 + 1.32 - (0.29 + 0.29) = 2.13$ gm. SO_3. The neutral sulphur elimination remained, as in the first experiment, wholly unchanged. Our patient was unfortunately during this feeding experiment allowed to do somewhat more severe work than in the previous experiment.[2] In attempt-

[1] The cystin used was snow-white, and consisted almost exclusively of perfectly formed hexagonal plates.

[2] Having expressed a desire to be a plumber, he was made assistant to the hospital plumber.

ing to provide for the extra amount of fuel called for by the work we increased his allowance of butter to 200 gm., but this resulted in a slight diarrhœa (three or four movements per day), and this is undoubtedly the reason why only 50 per cent of the cystin given was absorbed. The significance of the figures obtained is, however, unmistakable. Three grammes of cystin were absorbed, and the corresponding amount of sulphur was quantitatively eliminated as inorganic sulphates.

Before any serious attempt can be made to explain the curious behavior of persons having cystinuria toward protein sulphur and toward pure cystin, it would seem necessary to acquire more exact data.

Are there different degrees of cystinuria?

Most writers on the subject seem to assume that there are different degrees in the intensity of the disorder, but so far as we are aware no facts are known which can be accepted as proof in favor of such an assumption. It seems to us probable that the daily amount of cystin eliminated depends on the one hand on the amount of protein consumed, and on the other on the weight and size of the person. A child may consequently eliminate less cystin than a full-grown person, either because it eats less or because it weighs less, and in neither case would the result necessarily indicate a milder form of the disorder. Yet this question must be answered before any explanations can be attempted. For example, the "impaired oxidation" theory can clearly not alone be an adequate explanation if it should be found that the answer to the above question is negative.

If the daily amount of cystin eliminated had been as great on the starch as on the protein diet, then it would have been clear that the origin of the cystin is the sulphur coming from the general tissues. But the neutral sulphur values, as found in Tables I and II, indicate that on the non-nitrogenous diet the cystin is absolutely diminished, and relatively to the total sulphur is increased. The fact that the neutral sulphur remains greater than normal on a protein-free diet and relatively more prominent than on a protein-rich diet, taken together with the fact that (in our case) pure cystin does not pass through the system in unchanged condition, indicates clearly that *the cystin which is eliminated* is not absorbed as such from the intestinal tract. Our facts would rather suggest that the food sulphur which is eliminated as *sulphates* may be *absorbed* as *cystin*, and that it is the sulphur which is absorbed in larger or different complexes

TABLE III.

NITROGEN-FREE DIET.

Date. May.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	Neutral SO_3. "S_3."	In per cent of Total Sulphur.			Titrated acidity. c.c. $\frac{N}{10}$.	Total phosphates as P_2O_5. gm.	Remarks.
					S_1.	S_2.	S_3.			
8	1.40	0.52	0.10	0.78	37.3	7.1	55.6	207	1.30	
9	1.14	0.35	0.09	0.69	31.0	8.2	60.8	200	1.23	
10	0.98	0.30	0.08	0.60	30.5	8.0	61.5	170	1.10	
11	2.03	1.35	0.06	0.61	66.7	3.1	30.2	243	1.25	6 gm. pure cystin (from hair) given in the forenoon.
12	1.91	1.32	0.08	0.52	69.0	4.0	27.0	292	1.28	
13	0.96	0.29	0.11	0.57	29.8	11.2	59.0	277	1.39	
14	0.97	0.29	0.10	0.59	30.1	8.8	61.1	256	1.52	
15	0.95	0.32	0.12	0.51	33.5	13.1	53.4	221	1.25	
16	1.01	0.34	0.12	0.55	33.5	11.7	54.8	196	1.26	
17	0.97	0.29	0.13	0.55	29.7	13.2	57.1	178	1.18	
18	0.97	0.33	0.08	0.59	32.6	8.4	59.0	166	0.99	

TABLE III — *Continued.*

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. gm.	Urea. gm.	Ammonia. c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. May.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined nitrogen.
62.4	*125*		*14.0*	*289.0*	*1.77*	*0.34*							
8	1570	8.48	6.56	0.40	0.66	0.11	0.75	77.4	4.7	82.1	7.8	1.3	8.8
	12		*9.0*	*207.0*	*1.73*	*0.37*							
9	1475	5.74	4.15	0.29	0.64	0.12	0.54	72.3	5.0	77.3	11.1	2.1	9.5
	15		*6.2*	*183.0*	*1.61*	*0.36*							
10	1090	4.58	2.92	0.26	0.60	0.12	0.68	63.7	5.6	69.3	13.1	2.6	15.0
	12		*5.4*	*337.0*	*1.72*	*0.35*							
11	1380	4.31	2.59	0.47	0.64	0.12	0.53	60.0	10.9	70.9	14.8	2.8	11.5
	17		*4.9*	*416.0*	*1.59*	*0.35*							
12	1000	4.37	2.29	0.58	0.59	0.12	0.99	52.4	13.3	65.7	13.5	2.7	19.1
	10		*6.4*	*422.0*	*1.67*	*0.43*							
13	1650	5.06	2.96	0.59	0.62	0.14	0.75	58.5	11.6	70.1	12.2	2.7	15.0
	105		*7.3*	*362.0*	*1.63*	*0.41*							
14	2210	5.26	3.42	0.51	0.61	0.14	0.58	65.1	9.6	74.7	11.6	2.6	11.1
	125		*6.0*	*340.0*	*1.61*	*0.35*							
15	1150	4.73	2.79	0.48	0.60	0.12	0.74	59.0	10.1	69.1	12.7	2.5	15.7
	95		*7.9*	*314.0*	*1.53*	*0.36*							
16[1]	1965	5.53	3.71	0.44	0.57	0.12	0.69[1]	67.2	7.9	75.1	10.3	2.2	12.4
	10		*8.4*	*240.0*	*1.58*	*0.53*							
17	1680	5.70	3.91	0.35	0.59	0.18	0.67	68.7	6.1	74.8	10.4	3.1	11.7
60.3	*14*		*7.8*	*252.0*	*1.58*	*0.37*							
18	1190	5.50	3.69	0.35	0.59	0.12	0.75	67.1	6.4	73.5	10.7	2.2	13.6

[1] 4 gm. tyrosin given in the forenoon.

together with the sulphur derived directly from the tissues which the cystinuric individual is unable normally to convert into sulphates.

In view of the inexplicable difference between our results and those of Loewi and Neuberg it seems, however, useless to dwell on explanations and theories. More facts is what is needed.

A few other points of interest may be briefly mentioned here.

1. Loewi and Neuberg found, as stated at the beginning of this paper, that their patient did hydrolize and oxidize the cystin obtained from cystin stones, and that it was only the other kind of cystin (that found in urine or obtained from protein) which passed through the system unchanged. This difference is due, according to Neuberg, to a different chemical constitution of the two kinds of cystin.

The purely chemical findings of Neuberg concerning the two kinds of cystin have, however, not yet been verified by any other investigator, while decided doubts about their accuracy have been expressed by Rothera[1] and by Gabriel.[2] The former, working with Hopkins, says: "I do not feel the slightest doubt but that the specimen of calculus cystin dealt with by me was identical with hair cystin;" and the latter, after examining a sample of Neuberg's calculus cystin and comparing it with synthetical "isocystin" ($CH_2 . NH_2 . CH(SH) . COOH$) says: "Hiernach halte ich es für wenig wahrscheinlich, dass das Steincystin die Constitution des Isocystins besitzt."[3]

[1] ROTHERA: Journal of physiology, 1905, xxxii, p. 177.

[2] GABRIEL: Berichte der deutchen chemischen Gesellschaft, 1905, xxxviii, p. 641.

[3] After having already read the galley proof of this paper we received the June number of HOPPE-SEYLER'S Zeitschrift für physiologische Chemie, in which NEUBERG (and PAUL MEYER) publish two additional articles on cystin and cystein. From these it appears that NEUBERG no longer claims that calculus cystin is always different from protein cystin. On page 480 occurs the following paragraph:

"Keinesfalls kann das Cystin eines Steines ohne weiteres als verschieden vom Proteincystin betrachtet werden, sondern es bedarf stets der mikroskopischen Kontrolle. Diese ist aber beweisen."

He now states that only the calculus cystin, the crystals of which consist of *needles*, has a chemical constitution which is different from that of protein cystin.

After having read the above papers, one of us (F.) immediately examined a small stone recently passed by our patient. The stone was dissolved in hot, dilute ammonia-water, and the solution was allowed to evaporate in a desiccator containing sulphuric acid. The resulting residue consisted exclusively of typical hexagonal crystals.

2. The feeding experiments with asparaginic acid and with cystin described above showed that our patient was able to katabolize amido acids. In connection with the last feeding experiment, Table III, we tried one more amido acid, namely, tyrosin, 4 gm. of which was given to the patient on May 16th. The "undetermined" nitrogenous rest obtained on this and the following two days show that this amido acid also, in so far as it was absorbed, was destroyed or changed before its nitrogen reached the urine.[1] The absence of tyrosin from the urine was further established by means of Millon's reagent. All the urines of our patient gave a slight Millon's reaction, but this was no stronger than usual on the tyrosin day.

The slight Millon's reaction given by all the urines suggests the possibility that tyrosin was present in traces, especially in view of the fact that one or two investigators (Conti and Moreigne) claim to have detected the presence of tyrosin in cystinuria urines.

Parallel tests made with the urines and with tyrosin solutions indicated that the color reaction in the urine was not due to tyrosin. The color appeared immediately in the urine in the cold, while with the tyrosin solution heating was necessary.

Moreigne's test for the tyrosin as described by Simon[2] is moreover of doubtful value. He treated the cystin sediments with hydrochloric acid when the cystin crystals dissolved and the "rosettes" of "tyrosin" crystals appeared. The "rosettes" which he observed were probably the cystin hydrochloride. These resemble very much the tyrosin crystals, and they are frequently obtained when pure cystin crystals are treated with hydrochloric acid.

3. In connection with our two feeding experiments with pure cystin it was shown by means of sulphate determinations that none of the cystin fed passed unchanged into the urine. One peculiarity observed in connection with those experiments has not yet been mentioned. The cystin-sulphur was eliminated as sulphates, but the cystin-nitrogen was apparently not eliminated as urea. On the day following each cystin feeding, March 7th and May 12th, an unmistakable rise in the "undetermined" nitrogen was found. This rise was observed after the first cystin feeding, but in view of the result given by the sulphate determinations it was regarded as an accidental variation in one or another constituent that enters into this fraction of the urinary

[1] 0.293 gm. tyrosin subjected to a "urea determination" gave only 1.4 c.c. $\frac{n}{10}$ NH_3 corresponding to 2 mgm. nitrogen.

[2] *Loc. cit.* p. 52.

nitrogen. With the recurrence of a similar and greater rise in the undetermined nitrogen after feeding a larger quantity of cystin such an interpretation would seem improbable.

It is not impossible that this phenomenon may furnish the explanation as to why the nitrogen metabolism of our patient is not normal. The increased "undetermined" nitrogen eliminated at the expense of urea and ammonia, particularly on the protein-rich diet, may to a large extent be derived from those cystin groups whose sulphur is eliminated as sulphates.

4. The reader's attention is called to the fact that our patient eliminated decidedly more kreatinin and uric acid and neutral sulphur in the last feeding experiment, Table III, than in the second, Table II. During the second experiment he did very light work, and in fact for a part of the time was kept quite idle on a hospital ward. During the third experiment he was kept at work as a plumber's assistant. It seems quite remarkable and perhaps not altogether normal that such relatively moderate work could cause such an increase in the production of the above-named three constituents, but we are at a loss how to advance any other explanation of the facts observed.

NOTE. — While this paper was in the hands of the printer a private communication was received from Simon stating that he had just sent a brief note to the editor of the Zeitschrift für physiologische Chemie on the behavior of his cystinuria patient toward tyrosin. He also has obtained results which do not verify the findings of Neuberg and Loewi.

A STUDY OF THE COMPARATIVE EFFECTS OF SOLUTIONS OF POTASSIUM, SODIUM, AND CALCIUM CHLORIDES ON SKELETAL AND HEART MUSCLE.

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THE very numerous facts which have been published since 1875, when Merunowicz[1] showed the relation existing between the inorganic constituents of the frog's serum and the activity of the heart, indicate with a high degree of probability the essential importance of potassium, calcium, and sodium salts in the maintenance of the normal condition of muscle tissue. These salts, in order that they may be most efficacious, must exist in definite proportions in the solution surrounding the muscle, and in such amounts as to form a medium isosmotic with the tissue fluids. For the terrapin's heart, as an example, the proportions must approximate those in a Ringer solution of NaCl 0.7 per cent, $CaCl_2$ 0.026 per cent, and KCl 0.03 per cent.[2] The percentages of these salts may vary within limits depending upon unknown differences in the kind of muscle[3] and upon the condition in which a muscle may chance to be at a given time.[4]

In 1899 Loeb[5] called attention to the fact that the production of the specific effects of the salts in question is intimately connected with their being in a state of dissociation. Since then it has been generally agreed that the physiological changes produced are ionic effects. It is highly probable that anions,[6] as well as kations, influ-

[1] MERUNOWICZ: Arbeiten aus der physiologischen Anstalt zu Leipzig, 1875, x, p. 132.

[2] GREENE: This journal, 1898, ii, p. 82.

[3] ROW: Journal of physiology, 1903, xxix, p. 440.

[4] RINGER: Journal of physiology, 1887, viii, pp. 20, 288; MARTIN: This journal, 1904, xi, p. 103.

[5] LOEB: Ueber Ionen welche rhythmische Zuckungen der Skelettmuskeln bevorrufen, Festschrift für A. Fick, Braunschweig, 1899.

[6] LOEB: This journal, 1900, iii, p. 327.

ence the reactions of an immersed muscle, but the importance of the kations is both greater and more immediate. The method of their action is by no means clear. Loeb[1] considers the ions as forming definite chemical combinations with the living matter of the muscle so that there may be sodium-, calcium-, or potassium-proteid compounds. The proportion of these compounds formed depends upon the relative number of sodium, calcium, and potassium ions which leave the medium surrounding the muscle and diffuse into the tissue. If any one ion increases in numbers, it supplants a certain number of the ions of other ion-proteid compounds until the relative proportions of the latter are the same as those of the uncombined ions.

The impression given by Loeb's published statements on this point is that the substance of the muscle fibre is freely permeable to ions, but that the sarcolemma forms a more or less semipermeable membrane. In contrast to this stands the more definitely expressed opinion of Overton[2] that there is little if any diffusion into an inactive muscle. The latter is regarded as a complex system of structures of limited permeability surrounded by sarcolemma and perimysium of entirely different osmotic properties. The sarcolemma and connective tissue envelopes offer no resistance to the diffusion of most crystalloids in solution, but the muscle fibres themselves as long as they remain normal are impermeable, or nearly so, to the majority of inorganic substances. During the process of contraction, however, the permeability is altered so that diffusion takes place to an extent sufficient to produce physiological effects. Overton[3] states that when a muscle is immersed in a solution of electrolytes under such conditions that physiological effects are produced the concomitant diffusion does not follow the ordinary laws of diffusion, so that it is necessary to assume an active participation of the living muscle.

There is a general consensus of opinion that potassium salts in a bathing medium produce, or tend to produce, an inhibition of vital processes if they are present in sufficient quantities, but considerable differences of opinion exist with regard to the exact rôle played by the kations calcium and sodium. The investigation of the part they play has been prosecuted most vigorously in connection with contrac-

[1] LOEB: This journal, 1900, iii, p. 327.
[2] OVERTON: Archiv für die gesammte Physiologie, 1902, xcii, p. 115.
[3] OVERTON: Archiv für die gesammte Physiologie, 1904, cv, p. 207.

tion phenomena. In 1893 there appeared a research by Howell and Cooke [1] in which the authors undertook an investigation of the relative nutritive powers of serum albumin and serum globulin in the maintenance of the heart in activity. Their work led them to a special study of the sodium-calcium-potassium mixture on account of its remarkable power of sustaining the rhythmicity of the heart. A series of papers brought out under Howell's initiative culminated in his well-known theory of the relation of sodium, calcium, and potassium salts to the production of the heart beat. This theory [2] is the logical outcome of the fundamental work begun by Ringer.[3] It lays special emphasis upon the importance of calcium.

Seemingly opposed to this theory is the one of Loeb, which, based upon Biedermann's observation made in 1881 that a frog's sartorius will twitch in an alkaline solution of sodium salts, has been used to interpret the experimental results obtained from a great variety of rhythmically contractile tissues. Loeb's views [4] are of interest here only in so far as stress is laid upon the importance of the sodium ion in the production of rhythmicity. In 1900 Lingle [5] attempted to apply Loeb's views to the phenomena of the beating heart. He differentiated between agents which start a series of beats and those which maintain the series when once started, placing sodium among the former and calcium among the latter. The rhythmical contractions of the heart in 0.7 per cent saline are regarded as homologous to the twitches of striated muscle in the same solution. It is indeed desirable to establish a parallel in the reactions of the two tissues which, though alike in some respects, are nevertheless unlike in others. The inhibitory effect which calcium in physiological amounts exerts upon the twitches of skeletal muscle immersed in saline becomes an augmentor effect when this ion is applied to heart strips beating in saline.

A rapidly growing literature has made it strikingly evident that the reactions of a great variety of tissues to the same electrolytes differ markedly. In illustration of this may be cited the fact that potassium salts are less injurious to many plant cells than are the

[1] HOWELL and COOKE: Journal of physiology, 1893, xiv, p. 198.

[2] HOWELL: This journal, 1902, vi, p. 181.

[3] RINGER: Journal of physiology, 1883, iv, pp. 29, 370; 1884, v, p. 352; 1885, vi, p. 154; 1886, vii, p. 291; 1887, viii, pp. 20–288.

[4] LOEB: Ueber Ionen welche rhythmische Zuckungen der Skelettmuskeln bevorrufen, Festschrift für A. Fick, Braunschweig, 1899.

[5] LINGLE: This journal, 1900, iv, p. 270.

corresponding sodium salts; the reverse being true for muscle fibres.[1] Lillie[2] has shown that pure sodium chloride solutions produce rapid deterioration of the cilia of Arenicola larvæ, while Maxwell[3] has found it comparatively innocuous to the cilia lining the frog's œsophagus. Pure distilled water is generally agreed to be harmless to fresh-water organisms, but Bullot[4] has demonstrated that this is not true for the fresh-water Gammarus. Many other examples might be given. Cardiac and skeletal muscle present two important points of difference in their power of response to excitation. The former is characterized by a relatively long refractory period and by the fact that its contractions obey the "all or none" principle. The possibility is apparent that these tissues may likewise differ in their reactions to salts in solution. For this reason it has seemed desirable that a detailed study should be undertaken of the similarities and dissimilarities of the effects of sodium, calcium, and potassium chloride upon heart and skeletal muscle.

This work was done at the suggestion and under the direction of Dr. Howell, to whom the author is greatly indebted for ever constant interest and guidance.

The apparatus varied with the requirements of the experiments, but was always of the simplest description. The distilled water and the salts were prepared after the customary manner of this laboratory, as described by Greene.[5] The amount of the solutions used was twenty-five cubic centimetres at a time. In the majority of the tests the sartorius of the frog and heart strips from the ventricle of the terrapin were the tissues used. Experiments carried out on other muscles of the frog show that different muscles exhibit minor variations. Warm weather also modifies the results.[6]

Tone Changes.

Pure isotonic solutions. — When the sartorius muscle which has been carefully removed from the body of a frog is suddenly surrounded by a 1 per cent solution of potassium chloride (approximately isotonic with the body fluids), it exhibits a moderately rapid contraction

[1] Overton: Archiv für die gesammte Physiologie, 1904, cv, p. 207.
[2] Lillie: This journal, 1901, v, p. 56.
[3] Maxwell: This journal, 1905, xiii, p. 154.
[4] Bullot: University of California publications, Physiology, 1904, i, p. 199.
[5] Greene: This journal, 1898, ii, p. 82.
[6] Martin: This journal, 1904, xi, p. 103.

followed by a somewhat slower relaxation, both changes being readily followed with the eye, and one or both may be either regularly progressive or made up of a series of rapid twitches. The relaxation may not be complete. It may be marked by a small contraction remainder which slowly disappears. Or if the muscle happens to be in condition of slight contraction brought on by handling or otherwise, it may relax to a length greater than that before immersion. In any case, if allowed to remain in the solution, it continues in an extended condition, showing, however, a second very slight but constant variation in its length. Experiments in which the muscle is so arranged as to record this variation upon a slowly revolving drum give evidence that the initial potassium contraction is followed by an almost imperceptible lengthening during the first quarter of an hour, after which the muscle grows shorter.

This second contraction continues for about twenty-four hours, when it reaches a maximum. The immersed sartorius now relaxes at a somewhat more rapid rate than the previous shortening, until the writing-point has sunk far below the level of the starting-point. Extreme relaxation is reached from the fourth to the sixth day after immersion, when in nearly all cases putrefaction is well advanced. The details of a typical experiment are recorded in column 2 of Table I, in which, however, the changes in length due to the first potassium contraction are omitted.

The relatively rapid changes in length resulting from the first contact of the muscle with the solution, and which may be designated the "initial potassium contraction" are omitted for the present and will be discussed separately later on. A 2 per cent solution of potassium chloride gives qualitatively the same results as a 1 per cent solution. Miss Moore[1] has described a somewhat similar behavior of the gastrocnemius. When placed in strong solutions, an immediate strong contraction with occasional twitching occurs. This is followed by a slight relaxation, which sometimes amounts to one-half of the original contraction. Contraction then begins again and continues until rigor is complete. After rigor complete relaxation again occurs.

These changes in length as given in Table I follow in a general way the changes in weight which the muscle exhibits. These have been determined by Overton,[2] who states that the muscle may after twenty to twenty-four hours attain double the original weight, after which it

[1] MOORE: This journal, 1902, vii, p. 1.

[2] OVERTON: Archiv für die gesammte Physiologie, 1904, cv, p. 207.

loses again until it is reduced to one and one-half times the original weight. He states that during this time the muscle shows no changes in length, but remains in an extended condition. Since the changes

TABLE I.

Changes in the length of the sartorius muscle of the frog in isotonic solutions. Magnification 3 ×. The plus and negative signs refer to positions above and below the level of the starting-point respectively.

Time.	KCl 1%. Second contraction.	NaCl 0.7%.	$CaCl_2$ 1%
	mm.	mm.	mm.
Jan. 16, 10.00 A. M.	0.00	0.00	0.00
" " 10.30 A. M.	−1.25	−2.25	
" " 2.00 P. M.	0.00	−5.25	+40.50
" " 4.00 P. M.	+1.50	−6.00	+49.50
" 17, 9.30 A. M.	+10.50	−7.50	+29.50
" " 11.00 A. M.	+18.00	−8.00	
" " 12.00 M.	+19.00	−8.50	
" " 2.00 P. M.	+4.50	−9.25	+27.00
" " 3.00 P. M.	−2.25	−9.50	
" " 5.00 P. M.	−4.25	−10.00	+25.50
" 18, 10.00 A. M.	−6.50	−13.75	+24.25
" " 5.00 P. M.	−10.00	−16.00	+23.50
" 19, 10.00 A. M.	−12.25	−17.75	+20.75
" 20, 9.00 A. M.	−17.00	−22.50	+15.50
" 21, 10.00 A. M.	−23.00	−26.75	−6.00
" 22, 3.00 P. M.	−29.00	−33.00	−6.00
" 23, 9.00 A. M.	−29.00	−33.00	

shown in Table I might possibly be due to the increased weight of the sartorius disturbing the balance of the writing-lever, a number of experiments were made in which a weight equal to that of the muscle was added to the latter. No change in the position of the writing-point occurred. It required, in fact, a load more than six times that of the muscle to overcome to an appreciable extent the friction of the recording mechanism. Either Overton's frogs differ, in this respect, from

those used here or he overlooked these small changes, which roughly amount to only one-tenth of the muscle's length.

When immersed in a 0.7 per cent sodium chloride solution, the sartorius manifests a loss of tone which reaches its maximum extent, as in the case with potassium chloride, during the fourth to the sixth day. This is shown in the third column of Table I. Usually a rapid initial fall is followed by a more gradual one, which in turn becomes accelerated when putrefaction is well under way. The fall in tone is rarely absent. Out of seventy-six tracings taken at random it failed to appear in but one case, and in all of these the characteristic sodium twitches were present. It has been determined by Overton and Loeb that there is a slight gain of weight by the muscle immersed in an isotonic sodium chloride solution. According to Loeb [1] the gain varies from 6 to 8 per cent of the original weight during eighteen hours' immersion. The fact that the shortening of the muscle is not dependent upon changes in weight, which is evident in the case of potassium chloride, is more marked in sodium chloride, where a loss in tone accompanies a gain in weight. It is also shown by the behavior of the sartorius in calcium chloride. Solutions of the latter, isotonic with 0.7 per cent sodium chloride, produce during the first twelve hours a great increase in tone which disappears slowly. This is shown in the fourth column of Table I. According to Overton [2] there occur during the first fifty-five hours of immersion in a 1 per cent calcium chloride solution a slight loss in weight and then a gain.

In the investigation of the functional alterations taking place within the muscle, changes in length, or tone changes as they are here called, form a far more delicate indicator of such alterations than do changes in weight, but in turn are less delicate than the changes which can be observed to take place in the processes of contraction and relaxation caused by an electrical stimulus. If, for example, a sartorius muscle is immersed in a 1 per cent solution of potassium chloride, certain alterations take place during the first ten to twelve minutes. Periodical stimulation of the muscle reveals a rapidly declining power of the muscle to contract. If the tone changes only are observed, there may be witnessed the single preliminary potassium contraction or potassium twitches followed by a

[1] Loeb: Archiv für die gesammte Physiologie, 1899, lxxv, p. 303.

[2] Overton: Archiv für die gesammte Physiologie, 1904, cv, p. 207.

loss of tone, while changes of weight as stated by Overton cannot be demonstrated with certainty during this period.

The experimental results upon the tone of the sartorius obtained with pure solutions of sodium, calcium, and potassium chloride isotonic with the frog's tissue fluids, show that sodium chloride permits a relaxation of the muscle which merges gradually into the final lengthening due to the disorganization of the tissue. Calcium chloride produces a slow shortening, which persists until the final disorganization, when the tissue becomes stretched under the influence of the weight which it supports. Potassium chloride produces a moderately rapid shortening and relaxation. The relaxation merges into a slower lengthening, which in turn is not readily separable from the final lengthening due to the internal disintegration of the structure of the muscle. The fact that the sartorius exhibits a rapid contraction and relaxation followed by a slower shortening and lengthening may possibly be due to a mixture of two different contractile elements which, either in Grützner's[1] or in Bottazzi's[2] sense, enter into the formation of the sartorius of the frog. It is conceivable that the two elements may differ in their response to solutions of potassium chloride in such a way that the contraction and relaxation of one take place long before the other.

The effects of pure solutions of sodium chloride and calcium chloride on terrapin's heart and on frog's sartorius are quite similar as regards tone changes. In both muscles sodium chloride produces a loss and calcium chloride a slow increase in tone. The shortening produced by calcium in the heart is more vigorous than that shown by skeletal muscle, coming on more quickly, but being of shorter duration. In both relaxation occurs sooner or later. In one instance the sartorius began to relax six hours after immersion, while the heart had already begun to relax at the expiration of the first hour. But towards potassium solutions they present a marked disagreement. Heart strips taken from the ventricle of the terrapin or frog and placed in a 1 per cent solution show no stimulation effects, but manifest only a progressive loss of tone. This forms one of the most striking differences in the reactions of the two tissues in question. However, it should be stated at this point that a reference to the literature does not bear out this point very satisfactorily. Stiles[3] has

[1] GRÜTZNER: Recueil zoologique suisse, 1884, i, No. 4. Reference taken from Biedermann's Electrophysiologie, i, p. 111. Translated by Welby.

[2] BOTTAZZI: Journal of physiology, 1897, xxi, p. 1.

[3] STILES: This journal, 1903, viii, p. 271.

shown that for stomach tissue potassium chloride has a relaxing effect as long as the percentage is below 0.15, — higher doses causing a tonic contraction. Martin[1] corroborates these results for the terrapin's heart, but places the amount necessary at 0.23 per cent. "About 0.23 per cent potassium chloride appears to be the dose at which variations in tone do not occur in either direction." Greene[2] states that 1 per cent potassium chloride applied to heart strips beating previously in dilute serum resulted in one or two spasmodic contractions and then quiescence in a condition of tone. Lingle[3] has reported that a solution of potassium chloride, equimolecular with 0.7 per cent sodium chloride, always causes a tonic shortening in strips that are not beating. The author's results are not opposed to those stated above if the assumption is made that Martin's, Greene's, and Lingle's tests were not carried out on perfectly fresh strips. Fresh strips placed directly into a 1 per cent solution of potassium chloride always gave relaxation. One-half of the number of strips placed in 0.7 per cent saline for one, two, three, and four hours gave single beats upon transferal to potassium chloride. Previous immersion in Ringer for two to three hours gave rise to small contractions in the strip on subsequent treatment with potassium. Subjection to Ringer for two hours followed by a short bath in 8 per cent cane-sugar gave rise to a slow contraction and relaxation of the strip when immersed into 1 per cent potassium chloride, and these changes were of greater amplitude than when Ringer alone was used. Frequent stimulation of the strip with break-induced currents, while being subjected to Ringer solution for one-half hour was followed by a good contraction on subsequently exposing to potassium. These results were established in twenty experiments on strips taken from the ventricle of the common slider terrapin, Pseudemys rugosa, during the months of March and April. They can be accounted for by the assumption that the shortening produced by potassium is dependent upon the presence of calcium in the tissue in a particular form. If calcium is supplied by bathing the muscle in Ringer or by placing the muscle under conditions that permit the spontaneous conversion of calcium from an ineffective to an effective form, as has been supposed by Martin,[4] then the strip becomes capable of responding to the potassium solution.

[1] MARTIN: This journal, 1904, xi, p. 103.
[2] GREENE: This journal, 1898, ii, p. 82.
[3] LINGLE: This journal, 1900, iv, p. 265.
[4] MARTIN: This journal, 1904, xi, p. 103.

Combinations of two isotonic solutions. — The loss in tone of the sartorius which takes place when immersed in a 0.7 per cent saline solution is very slightly if at all influenced by small additions of potassium chloride. In this respect there is a point of difference between skeletal and cardiac muscle. Greene states that terrapin heart strips in 0.7 per cent sodium chloride plus 0.03 per cent potassium chloride almost always show excessive loss of tone. If the potassium is added to the sodium as a 1 per cent solution, then the mixture remains isosmotic to the tissue fluids, but the solutions become mutually diluted. If, then, while the percentage of potassium becomes greater and the percentage of sodium becomes less, the effects of these combinations on the sartorius muscle are noted, it will be observed that among the first effects of the potassium is the suppression of the c'.aracteristic sodium twitches. With a further increase of the potassium chloride a phenomenon characteristic of the potassium begins to appear, namely, the initial potassium contraction. Small at first, this potassium contraction grows as the potassium content of the mixtures is made greater. A fresh vigorous sartorius first washed in saline may respond with a minimal potassium contraction if submitted to a solution consisting of 0.7 per cent saline plus 0.04 per cent potassium chloride, although sudden immersion into saline alone produces no effect. By first immersing into saline the error which might arise as the result of closing the demarcation current is avoided.

The minimal amount of potassium necessary in a mixture of 0.7 per cent sodium chloride plus 1 per cent potassium chloride in order to produce the second shortening which the sartorius may exhibit is not easily determined. Three muscles in solutions containing less than 0.04 per cent potassium, after a preliminary fall, began to shorten at intervals varying from six to twenty-four hours after immersion; but six others showed only a loss in tone. All of twenty-five muscles placed in solutions containing more than 0.04 per cent potassium gave rise in tone.

If in a similar manner there are added gradually increasing amounts of a 1 per cent solution of calcium chloride to 0.7 per cent saline, the loss of tone in the sodium chloride is replaced by an increase in tone due to the calcium. The latter in minute doses is not so efficient in causing a shortening of the muscle as is potassium. Beginning with an amount as low as 0.05 per cent in saline, the sartorius always showed some contraction, although the variations in the extent of the short-

ening were considerable. A short preliminary fall was often observed, arising from the fact that the latent period of the calcium effect is long, and during this time the muscle lengthens under the influence of the attached load. Calcium present in saline in amounts less than 0.01 per cent always permitted the muscle to relax. Like potassium, but even more readily, it inhibits the sodium twitches.

A comparison of the effects of combinations of the chlorides of sodium and calcium on heart strips and skeletal muscle shows that the tone changes produced are in general much alike in the two tissues. Calcium, as soon as the amount exceeds that in a Ringer mixture, antagonizes the relaxation which pure saline permits. When it forms as much as 0.05 per cent of the mixture, it gives rise to a slow shortening of the muscle. Heart strips respond much more quickly to calcium than do skeletal muscles.

Mixtures of sodium chloride plus increasing doses of potassium lead only to progressive elongation in terrapin heart strips as long as they remain in a fresh condition, while such solutions will produce the characteristic potassium contraction in the animal's skeletal muscles. The rapidity with which potassium produces a contraction in skeletal muscle stands in contrast to the slow shortening due to calcium.

Combinations of the three solutions. — In studying the effects of mixtures of the three solutions — sodium, calcium, and potassium chloride — in varying proportions on the sartorius of the frog, it is convenient to begin with a Ringer solution in which the amounts exist in physiological percentages, or nearly so. The Ringer mixture permits an elongation of the muscle under the influence of the extending weight very much as in simple saline, but the elongation is, perhaps, not so great. Variations in the state of tone are readily produced by proper changes in the percentages of the three constituents. Observing the precaution that the mixture as a whole remain approximately isotonic with the tissue fluids, it may be stated that a relative increase of the calcium over the other ions produces an increase in tone. That a relative increase of potassium leads to diminished tone unless the preponderance is excessive. An increase in the relative proportion of the sodium ions leads to diminished tone as in pure saline. The effects upon ventricular heart strips from the terrapin resemble precisely as regards tone those of the frog's sartorius except in the case where potassium is present in large amounts. The preliminary potassium contraction does not take place, and as

long as the ventricular strip remains fresh only diminished tone is observed.

The effects after previous treatment. — If the actions of the salts in question are due to the diffusion of ions into and out of the muscle, it is to be expected that preliminary treatment of the muscle so as to change its ion content or perhaps change only the surface layer would affect the results obtained from subsequent treatment with the chlorides of sodium, calcium, or potassium. With this possibility in view the sartorius was given baths in 7.7 per cent cane-sugar solutions for varying lengths of time, after which followed immersions into isotonic solutions of the three chlorides, singly or in combination. The data derived from more than one hundred and fifty experiments bearing on this point may be summarized as follows: A solution of cane-sugar applied to the sartorius of the frog produced in the majority of cases no changes in tone for many hours. Eventually an increase in tone was obtained. Immersion into 0.7 per cent saline for from one to five hours altered the muscle so that the application of cane-sugar gave an immediate increase in tone in thirty-eight out of forty-three muscles. The addition of calcium chloride in physiological amounts to the sodium chloride rendered an immersed muscle more resistant to subsequent application of cane-sugar. The shortening produced by the sugar was to some extent at least readily reversed by plunging the muscle into pure solutions of sodium, calcium, or potassium. The subsequent behavior of the muscles if allowed to remain in these solutions was much as if the preliminary bath in sugar had not been given. Sodium chloride caused continuous relaxation; calcium chloride a more vigorous contraction and potassium chloride, also in time produced a slow contraction and relaxation. Brief preliminary treatment with sugar did not destroy the first potassium contraction, but longer exposure prevented its occurrence.

If after an hour's bath in sugar the sartorius is transferred to a mixture of cane-sugar and varying amounts of sodium chloride, the changes in tone, if any at all are produced, are small and in the majority of cases in the direction of relaxation. Similarly, transferal after an hour's sojourn in 8 per cent cane-sugar to mixtures of cane-sugar plus calcium in varying amounts causes a shortening of the muscle when the calcium is present in sufficient amount to produce any effect at all. If small amounts of potassium chloride, *i. e.*, physiological doses or less, are added to the sugar solution, they apparently have no effect on the tone changes. Comparatively large

amounts in sugar allow a preliminary relaxation. The tone changes produced by sodium or potassium under these conditions are almost insignificant when compared with those produced by calcium.

The results of the experiments taken altogether seem to indicate that approximately isotonic solutions of cane-sugar produce no tone changes until after long immersion or when there is reason to suppose that the muscle has begun to undergo deterioration. The sugar in its action produces a stage during which the sartorius is more sensitive in its reactions to sodium, calcium, and potassium chloride, succeeded by a later stage during which this sensitiveness is so reduced that it falls below normal.

A comparison of the effects produced by a sugar solution on the frog's sartorius with those produced in terrapin heart strips shows in general much similarity. Heart strips show a loss in tone for an hour or less followed by an increase, while the sartorius may elongate when first immersed, but the development of tone is readily produced. If calcium chloride is added to the sugar solution after the heart strip has been immersed in it for some time, it shows a tendency to go into excessive tone. The sartorius under like conditions also develops tone. If potassium chloride is added in small quantities, it either exerts no effect or produces an increased tone in terrapin heart strips. In the sartorius there is also the production of tone, although it may be of much slower development. In both ventricular strip and sartorius the increased tone due to the immersion in sugar is reversed by applying 0.7 per cent saline.

The Potassium Contraction.

Since the discovery by Bernard and Grandeau[1] that potassium salts in suitable strengths call forth contractions of skeletal muscle, this fact has been corroborated by many observers. Among these may be mentioned Zenneck,[2] Grützner,[3] Zoethout,[4] and Overton.[5] Grützner, finding a 4 per cent solution ineffective when applied to a motor nerve, got a prolonged contraction by applying the same solution to a curarized gastrocnemius. Zoethout describes briefly

[1] Bernard and Grandeau: Centralblatt für die medicinischen Wissenschaften, 1864, p. 183.

[2] Zenneck: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 21.

[3] Grützner: Archiv für die gesammte Physiologie, 1893, liii, p. 83.

[4] Zoethout: This journal, 1902, vii, p. 199.

[5] Overton: Archiv für die gesammte Physiologie, 1904, cv, p. 207.

a series of contractions obtained by alternately applying potassium and calcium chloride to a gastrocnemius muscle, and calls attention to the antagonistic effects produced by the potassium and calcium respectively. Overton records the changes in the weight of a frog's sartorius when subjected to solutions of potassium chloride. No changes in weight during the first ten to twelve minutes could be determined with certainty.

The number of contractions evoked by immersions into potassium chloride is a small one. Alternation of potassium chloride with

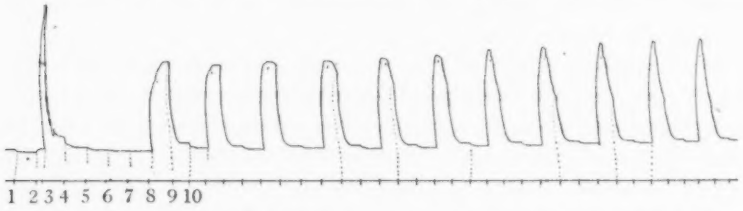


FIGURE 1. — Portion of a curve showing contractions of the sartorius muscle of a small bull-frog obtained by immersing into KCl solution. Temperature, 25°. At 1, immersion into NaCl; solution removed at 2; 3, immersion into KCl; 4, into NaCl; 5, into KCl; 6, into NaCl; 7, in $CaCl_2$. One minute each, with intervals of same duration. Solutions used were 0.7 per cent NaCl, 1 per cent KCl, and 1 per cent $CaCl_2$. The small dot near the top of each contraction indicates removal of K solution.

sodium chloride increases the number; an alternation of potassium chloride with calcium chloride gives a still greater number of contractions, and the application of the three chlorides in rotation gives the best results of all. This procedure when carried out on the sartorius of the frog gives a series of contractions like that produced by periodic electrical stimulation. The series shows sometimes one or more preliminary contractions of diminishing height analogous to the "introductory contractions," then a series of increasing height comparable to the "staircase," and finally a series of diminishing height as in "fatigue." A portion of such a curve is shown in Fig. 1, which was obtained from the sartorius of a small bull-frog. The solutions used were 1 per cent potassium chloride, 0.7 per cent sodium chloride, and 1 per cent calcium chloride, into each of which the muscle was immersed one minute at a time with intervening rests of the same duration when the muscle hung suspended in the air. At 1 the muscle was immersed in sodium chloride, which was removed at 2. At 3 the immersion was into potassium chloride. A rapid contraction followed, marked by irregular twitches on the descending limb.

At 4 the solution was replaced by sodium chloride. At 5 the muscle was again placed in potassium chloride, at 6 in sodium chloride, and at 7 in calcium chloride, and then once more, at 8, in potassium chloride. The contraction beginning with 8, and the remaining members of the series were obtained by applying potassium, sodium, and calcium in rotation.

There is, therefore, a great difference noticeable in the character of the first contraction and all that follow, — for in the latter the movement is slower and smoothly progressive; *i. e.*, the myogram shows no irregular twitches. This difference is a constant one. When the temperature is comparatively low, the muscle a vigorous one, and the subjection to the potassium chloride solution of brief duration, the muscle may respond several times to the application of the potassium, but usually there is but a single response, as shown in the accompanying curve. Whether left in the solution or withdrawn so as to hang in the air, the sartorius relaxes completely or nearly so. The behavior of the muscle when left in the solution for several days has already been described. The irritability disappears within a few minutes so that the muscle responds to neither mechanical nor electrical stimulation. Treatment with a bath of either sodium or calcium chloride restores the ability of the muscle to respond to potassium. But the series of contractions produced by an alternation of potassium and sodium differs decidedly from that produced by an alternation of potassium and calcium. The former is of shorter duration, the individual contractions of rapidly lessening amplitude, and complete relaxation to the base-line follows each shortening. In the latter the series is a longer one, but the muscle relaxes incompletely after each contraction, so that the lever rises as in contracture until the individual contractions become very small. An alternation in a similar manner of calcium chloride with sodium chloride produces no visible changes in the muscle. A series of contractions of maximum duration can be obtained only by the employment of all three solutions, — potassium, sodium, and calcium chlorides. Whether the calcium and sodium are applied separately or at once in a mixed solution matters little, except that it is better to use a mixture of 0.7 per cent sodium chloride with little calcium than a mixture of 1 per cent calcium chloride plus a small amount of sodium. The alternation of potassium chloride and a Ringer mixture also gives a good series.

As has been stated before, of the series so obtained the first contraction always (and sometimes the first two or three) differs in

character from the remainder. It possesses by far the shortest latent period which in fifteen determinations averaged 0.26 second — the extreme values varying from 0.045 second to 0.54 second. In contrast to this the latent period of the remainder of the series, as shown by seventy-one determinations made on eight muscles, equalled 0.8574 second on an average. The extreme individual values were 0.546 and 1.092 seconds. The method of obtaining the time relations of the contractions produced by the potassium chloride was the following: A drum driven at a uniform speed of five and one-half millimetres per second by an electric motor was marked by vertical lines so placed that the distance between them represented intervals of ten seconds each. The lower end of a sartorius muscle was attached to an L-shaped glass rod, while the upper end was attached to one end of a recording lever. As the drum revolved, the writing-point passed in succession the vertical lines on the drum, and at the moment of crossing a beaker holding about twenty-five cubic centimetres potassium chloride or other solution to be tested was quickly brought up so as to surround the muscle. The figures given for the latent period are, therefore, too large, as no correction was made for the reaction time, for the time required to bring the solution up around the muscle, or for variations in the size of the muscle. But they serve the purpose intended, giving qualitatively the changes produced in the reactions of the muscle under the influence of the three electrolytes.

The rapidity of shortening of the first contraction is strikingly greater than that of the others, reaching a maximum in 6.08 seconds when relaxation sets in, even though the muscle remains immersed in the potassium chloride. Usually the solutions tested were allowed to act upon the muscle ten seconds at a time, with intervening pauses of like duration during which the muscle hung in the air. Under such conditions the layer of fluid clinging to the surface of the muscle made the immersion equivalent to one twice as long. And, as a matter of fact, it was found that the exact time of removal of a solution could be varied through a considerable range without affecting the result, but the time of application had to be exact.

The time required by a muscle to reach its maximum of shortening when exposed to a solution of potassium chloride depends upon the immediately preceding treatment to which the muscle has been subjected. In a series obtained by alternately immersing the muscle into 1 per cent potassium chloride for twenty seconds and a Ringer solu-

tion for sixty seconds, it was found that the average time of shortening after the first contraction equalled 7.755 seconds. This average expresses the results of twenty determinations made on four muscles. If in place of alternating potassium chloride and Ringer, potassium, calcium, and sodium — or potassium, sodium, calcium, and sodium — are alternated within the same time limits, then the period of shortening grows to more than twenty seconds. This is the average of forty-eight determinations made on two muscles.

The first relaxation of a potassium contraction series is apparently uninfluenced by immersion in Ringer, sodium, or calcium. It runs its course, reaching the base-line in 50 per cent of the observations in about thirty seconds. In the remaining 50 per cent a small contraction remainder was still present at the end of thirty seconds, which although gradually diminishing continued for more than sixty seconds. All relaxations after the first are noticeably influenced by immersing the muscle into 0.7 per cent saline, 1 per cent calcium chloride, or 8 per cent cane-sugar. These agents all produce an augmentation of the rate of lengthening. In their effectiveness in producing this result they stand in the following order: sodium, calcium, and sugar. This may be judged by the completeness of the relaxation which results in a given time. Thus it was found that in sodium chloride the muscle relaxed 91.27 per cent of the height of contraction in ten seconds; 97.11 per cent in twenty seconds. When sodium chloride was replaced by 1 per cent calcium chloride, the lengthening in ten seconds was only 76.17 per cent; in twenty seconds, 92.11 per cent.

Relaxation in sugar takes place at a still slower rate, the amount varying according to the previous treatment which the muscle has undergone. If, for instance, a sartorius is repeatedly immersed in the following solutions in the order given, — K, sugar, Ca, sugar, K, sugar, Na, sugar, etc., — it will be found that the relaxation of a potassium contraction is altered regularly as a calcium or a sodium bath precedes the immersion into potassium. When the immersion into potassium has been preceded by a bath in calcium chloride, the relaxation in sugar equals 57.32 per cent of the total amount in ten seconds; 84.50 per cent in twenty seconds. When a bath in sodium chloride has preceded the potassium contraction, then the relaxation in cane-sugar equals 40.88 per cent in ten seconds; 74.12 per cent in twenty seconds. The effect of the calcium bath is, therefore, not removed by washing for an equal length of time in cane-sugar, and

similar changes can be detected in the height of the contractions and in the latent periods. The potassium contractions preceded by a calcium bath are of less height (about 6 per cent), while the latent period is about doubled. The effect of the calcium chloride, supplied as it is in great amount, is not favorable to the potassium contractions, — a result directly the reverse of that reached for small amounts. It should be stated, however, that the quicker relaxation in cane-sugar after a previous bath in calcium in contrast to a previous bath in sodium does not hold true if the muscle instead of being transferred to sugar remains in the potassium chloride. Under these conditions the relaxation, after a previous bath in calcium, is indefinitely prolonged.

Similarly it can be shown that preliminary treatment of the muscle with cane-sugar solutions for varying lengths of time just previous to immersions into potassium chloride alters the results. The latent period is lengthened and the height of the contraction is diminished. Preliminary treatment with potassium chloride produces the same results more rapidly. If a muscle is made inactive to potassium by alternately placing in potassium and sodium, it may, when treated to long baths in 0.7 per cent saline, show considerable recovery. In one such experiment in which the sartorius was subjected to sodium chloride for two, five, eight, ten, twenty, and forty minutes, it was found that the ability of the muscle to contract was restored at the end of five minutes, but the contraction was barely visible. The power of the muscle to shorten increased with the duration of the immersion in sodium chloride, until at the end of forty minutes the contraction reached $66\frac{2}{3}$ per cent of the height of the original contraction. Twenty seconds' sojourn in potassium chloride again destroyed the power of the muscle to contract. A Ringer solution under similar conditions can restore the muscle within one minute.

Based on the theory of the diffusion of ions into and out of the muscle, the above experiments may be explained as follows: Potassium produces a contraction followed by a relaxation in the sartorius when the two kations, sodium and calcium, are present in physiological amounts. If potassium and sodium solutions alternate so that the calcium present in available form diffuses out, then potassium rapidly becomes ineffective. If potassium and calcium solutions alternate so that sodium ions diffuse out, then potassium also becomes ineffective in producing further contractions, for the muscle enters into a state of rigor. It has been shown by many observers

that if a muscle is placed in potassium chloride the irritability, contractility, and conductivity rapidly disappear. Within certain limits washing with sodium chloride, calcium chloride, Ringer, or sugar solutions will re-establish contractility to varying degrees. After longer exposure this is impossible. Washing with Ringer supplies immediately the kations calcium and sodium. Alternating potassium with cane-sugar permits potassium contractions only during the time required for the ions of the muscle to diffuse out, and sugar has no power of restoring potassium contractions. Subjection of the muscle to calcium chloride produces definite changes that cannot be obviated by mere bathing in cane-sugar solutions for the same length of time. These changes manifest themselves, upon applying potassium, in an enormously prolonged contraction. Neither can a bath in 0.7 per cent saline obviate the changes produced by calcium. A muscle which has lost its power of responding to potassium by being alternately immersed into potassium and sodium will within one minute again respond vigorously if so minute a quantity of calcium as is present in a Ringer solution be supplied.

From a consideration of the phenomena exhibited by the sartorius when immersed in isotonic solutions it seems that the reactions of the muscle are influenced in two ways: first, by the mere fact that one solution is diluted by the presence of another, which thus delays the typical effects; second, the solutions in question have specific effects upon the contractile tissue, which may be alike or may be antagonistic. It can readily be inferred that the concentration of the potassium chloride solutions exercises an effect upon the contraction called forth. In one experiment the sartorius was submitted alternately to a 1 per cent solution of potassium chloride and Ringer until a series of contractions was well established. The employment of a 0.5 per cent solution instead of the 1 per cent effected a diminution of 55 per cent in the shortening. Change to a 10 per cent solution produced a contraction higher than any other of the series, but the deleterious effects were equally marked. When potassium and sodium or potassium and calcium are mixed in various proportions, but in such a way that the mixture always contains 1 per cent of potassium, and these mixtures are applied alternately with Ringer to the sartorius, they rapidly destroy the irritability of the preparation. The thirty-six tests thus made indicate that calcium is somewhat more effective than sodium in preventing a potassium contraction in a fresh muscle, but that when potassium is at all effective the mixture

of potassium plus calcium favors prolongation of the contracted state.

The rapid contraction and relaxation of the frog's sartorius in response to a 1 per cent solution of potassium chloride offers an interesting problem. It can very readily be shown that the normal living condition of the muscle is essential, for anæsthesia produced by ether vapor destroys the potassium contraction as quickly as it destroys the ability of the muscle to respond to electrical excitation. Furthermore, exhaustion of the muscle in 0.7 per cent saline by repeated electrical stimulation prevents the action of the potassium. Only a few experiments were made in this respect, but they indicated that the diminution in the height of the potassium contraction varied with the amount of fatigue of the muscle. This is only an extension of the results of experiments detailed above in which it was shown that previous exposure of the muscle to potassium, calcium, sodium, or sugar altered the normal condition, so that the response to potassium was modified. These substances are injurious to the muscle either *per se* or indirectly by allowing diffusible constituents to leave the muscle, or they are injurious in both ways. In the order of their injuriousness, if supplied alone, they seem to stand,—potassium chloride, calcium chloride, and sodium chloride. It requires but a short sojourn in potassium chloride, a longer one in calcium chloride, and a relatively very long exposure to sodium chloride to destroy the effectiveness of subsequent applications of potassium. Stimulation of the muscle with break-induced currents while submerged in these solutions shows the same order in the disappearance of excitability.

It seems obvious, therefore, that a 1 per cent solution of potassium chloride can produce its characteristic effects only when the muscle is in normal condition. There is a good deal of evidence that the maintenance of the latter is dependent, among many other conditions, upon the presence of the ions sodium and calcium, and the difference in the results obtained by alternating potassium and sodium, on the one hand, and potassium and calcium, on the other, is very significant. In the alternation of potassium chloride and sodium chloride the calcium ions diffuse out of the muscle, and it is conceivable that the loss of contractility is proportional to the loss of calcium ions. When calcium chloride and potassium chloride are alternated, sodium ions leave the muscle, which then promptly goes into a state resembling contracture.

These facts seem to point to the conclusion that potassium pro-

duces a contraction by an interaction involving calcium, and not, as is implied in the publications of Zoethout,[1] by the combined effects of an increase in the potassium content and a removal of the inhibitory influence of calcium. Furthermore, if the calcium present in the muscle inhibits the tendency of the sodium to produce a shortening, and if furthermore the access of potassium by antagonizing the calcium unmasks the tendency of sodium to produce a shortening and so induces the "potassium contraction," then it is to be expected that an alternation of potassium and sodium would call out a long series of contractions or at least a condition of contracture. But this is not the case. If, on the other hand, it is the calcium which is especially involved in the potassium contraction, then an alternation of potassium chloride and calcium chloride should bring out a series of contractions and tendency to contracture, which as a matter of fact actually occurs. Moreover, when after an alternation of potassium chloride and sodium chloride the potassium is no longer able to evoke contractions of the muscle, a supply of calcium restores to its full extent the power of the muscle to respond.

Additional evidence of the importance of calcium to the muscle is the fact that the application of reagents that are precipitants of calcium, like sodium sulphate or sodium oxalate, destroys the ability of the potassium solution to produce contractions. A mixture of 0.7 per cent saline and a saturated solution of sodium oxalate in proper proportions induces contractions which to all appearances are like those produced by potassium chloride. It is very tempting to assume that the method of action of both is the same. Martin[2] in his investigation on terrapin heart strips was led to the hypothesis that there is a spontaneous conversion of calcium from an ineffective to an effective form, particularly under the influence of sodium chloride. That a similar conversion may take place in skeletal muscle is made probable by immersing a sartorius alternately into potassium chloride and sodium chloride until potassium no longer produces contractions. If the muscle is then allowed to remain in 0.7 per cent saline for ten or fifteen minutes, it is found that the muscle has regained to some extent its power of responding to potassium. It is not inconceivable that when potassium is suddenly supplied to the muscle in great quantity it may, in common with the oxalate acceler-

[1] ZOETHOUT: This journal, 1902, vii, p. 320.

[2] MARTIN: This journal, 1904, xi, p. 103.

ate, the conversion of calcium from an ineffective to an effective form and thus produce a sudden contraction.

Potassium chloride stimulates the muscle, but not in the ordinary way, for the contractions produced are not propagated along the length of the fibre. This is shown in the relations between the height of the contractions produced and the extent of the immersion. Table II gives the results of a typical experiment. The extent of immersion was only approximately that of the figures given.

TABLE II.

The relation of the height of contraction to the extent of immersion. The sartorius muscle of the frog alternately immersed to varying extent in 1 per cent potassium chloride and a Ringer solution. Magnification 3 ×.

Extent of immersion.	Height of contraction.	Calculated height.
	mm.	mm.
About $\frac{3}{4}$	$21\frac{1}{2}$	24
About $\frac{1}{4}$	9	8
About $\frac{1}{3}$	13	$10\frac{2}{3}$
About $\frac{3}{4}$	28	24
$\frac{4}{4}$	32	

If a sartorius is fastened at its centre in such a way as to hold it firmly but so as not to prevent the passage of the wave of contraction called out by electrical or mechanical stimuli, and the free ends of the muscle are connected with recording levers, it will be found that only that half of the muscle contracts that is subjected to the potassium. If the one end of a fresh muscle under such conditions has been injured so that quickly plunging into 0.7 per cent saline will close the demarcation current, it will be found that the impulse thus originated sweeps over the entire muscle.

It has already been stated that the potassium contraction fails in fresh strips of the terrapin heart. Both cardiac and skeletal muscle show greater sensitiveness to the action of salts after exposure to conditions which are not strictly normal. The contraction which results, therefore, after special treatment of heart strips may be analogous to the contraction in striated muscle, but in its various phases it is much more sluggish.

The Fibrillary Twitches in Sodium.

Since 1881, when Biedermann[1] first reported that immersion of striated muscle in an alkaline solution of sodium salts calls forth fibrillary twitches, this fact has been verified repeatedly. In the experiments upon which this paper is based these characteristic sodium twitches were produced in practically 100 per cent of the number of sartorii placed directly into saline. The power of calcium chloride and of potassium chloride to inhibit the twitches was fully corroborated, both when small and when large additions were made to the solution. With minute doses the calcium acted rather more promptly than the potassium. It is stated that the fibrillary twitches may continue for days at a sufficiently low temperature. Skeletal muscle differs in this respect from heart strips of the terrapin's ventricle, which according to Martin reach sodium chloride exhaustion in about one hour and fifty minutes. Both the twitches of the sartorius and the beats of the heart strips are promptly inhibited by large doses of potassium or calcium, for example, by a 1 per cent solution. But here again a difference is to be noted. In minute doses the sartorius twitches are indeed inhibited by calcium or potassium, and the latter may also inhibit the heart, but calcium in suitable amounts improves strikingly the beat of the heart. The effect of diluting with cane-sugar the saline solutions into which the heart strips are immersed has been described by Howell.[2] The behavior in this case is similar to that observed in the muscle. The twitches of skeletal muscle and the beats of the heart strips do not begin promptly, but have a latent period, which is very short and often absent in the sartorius, but according to Martin averages forty minutes in the terrapin heart.

The results obtained by partial immersion of the sartorius into 1 per cent solutions of potassium chloride led to a repetition of the experiments with saline solutions. The simplest way in which this can be done is to place the muscle on the edge of a glass slide in such a way that the vertical half hangs down into 0.7 per cent saline, while the horizontal half rests on the slide in a pool of Ringer's solution. In these experiments the twitches were confined for varying lengths of time to that portion immersed in the saline, before the more vigor-

[1] Biedermann: Electrophysiologie, i, p. 113. Translated by Welby.

[2] Howell: This journal, 1902, vi, p. 181.

ous twitches spread to or involved the half immersed in the Ringer. This experiment was carried over to strips taken from the terrapin's ventricle with similar results. The method of the experiment, however, was slightly altered. Two beakers were placed side by side, one filled to the brim with a Ringer solution and the other with 0.7 per cent saline. Long strips from the ventricle were hung over the adjoining edges of the beakers so that each half of the strip dipped into one of the solutions. Under these conditions the beats began in the end in sodium chloride, and for a short time were limited to this end. As they grew in intensity they involved more and more of the portion of the strip in the Ringer solution until the entire strip was active, — the sodium chloride end, however, initiating the beat. The period of transition from partial to total activity of the strip was often marked by an independence of the rate of the beat of the two ends, so that the sodium chloride end beat three or two times to each beat of the end in the Ringer.

The Contraction Produced by Calcium.

Calcium in percentages above normal in solutions of sodium chloride or Ringer produces marked increase in the tone of both sartorius muscle and ventricular heart strips.

The slow contraction passing into an equally slow relaxation when the tissues in question are placed in 1 per cent solutions is accelerated in both by previous immersion into 8 per cent cane-sugar. That the calcium contraction is localized to the portion immersed in the solution can be demonstrated as in the case with potassium chloride. The following experiment was performed. A strip from the terrapin heart was lightly tied at the middle and fastened immovably to a glass standard. The free ends of the strips were connected with recording levers. One end was then subjected to 0.7 per cent sodium chloride; the other end to 1 per cent calcium chloride. The latter had just completed the slow contraction and relaxation resulting from the immersion into calcium chloride when the beats set up in the saline end began to pass the ligature. The curve traced on the drum by the calcium end of the muscle now showed a marked increase in tone, and exhibited irregular waves formed by groups of contractions. The saline end of the strip showed only progressive loss of tone.

THE BEHAVIOR OF THE SARTORIUS WHEN STIMULATED PERIODICALLY.

If the sartorius muscle of the frog while immersed in a 1 per cent solution of potassium chloride is stimulated once per minute with maximum break-induction shocks, it manifests a rapidly decreasing series of contractions. It is convenient in this and all similar experiments to permit the solution in question to bathe the muscle for fifty-five seconds in every minute. The remaining five seconds give ample time to remove the beaker containing the solution, to give the stimulus, and replace the solution. A series thus obtained consists of contractions varying from one to ten in number, progressively declining in height. After each contraction there is complete relaxation to the base line. The series of contractions becomes longer when the potassium solution is diluted with 0.7 per cent saline, and grows in length as the latter forms more and more of the mixture.

If the sartorius in a similar manner is submitted to a 1 per cent solution of calcium chloride, the resulting curve is quite different. In one experiment the height of the contractions diminished from forty millimetres to three millimetres during the first nine minutes. The diminution thereafter was much slower, so that in three hours the contractions were still one millimetre high. In the mean while certain tone changes had taken place which raised the base line to a height of twenty-four millimetres in one and one-half hours. The tone was then again lessened for an hour and a half, when the original level was reached. The entire curve therefore consisted of nine initial contractions, rapidly diminishing in height, followed by a series of small contractions superimposed upon a tone curve of three hours' duration. The superimposed contractions were executed rapidly with equally rapid relaxations, which gave the impression of an internal support. In describing the tone changes following immersion into a 1 per cent solution of potassium chloride it was found convenient, in order to explain the phenomena, to assume the presence of two contractile elements. A similar assumption can be made in the case of calcium chloride where the rise in tone can be regarded as due to the reaction of the more sluggish element.

Stimulation of the sartorius immersed in a 0.7 per cent saline solution gives a decidedly longer series of contractions than when the immersion is into potassium chloride or calcium chloride, but not so good a series as in a Ringer solution. Sodium chloride, though less

injurious than the potassium or calcium chloride, nevertheless has an effect. It leads to a state resembling sodium chloride exhaustion in heart strips. When in this condition physiological amounts of calcium chloride restore, sometimes to a remarkable degree, the vigor of the contractions, and the action of calcium in this respect stands in contrast to the inhibitory effect it possesses on fibrillary twitches. In Fig. 2 this difference is illustrated. The same amount of calcium applied after the same length of time stops the twitches in an unstimulated sartorius, while it restores the contractions of the stimulated muscle.

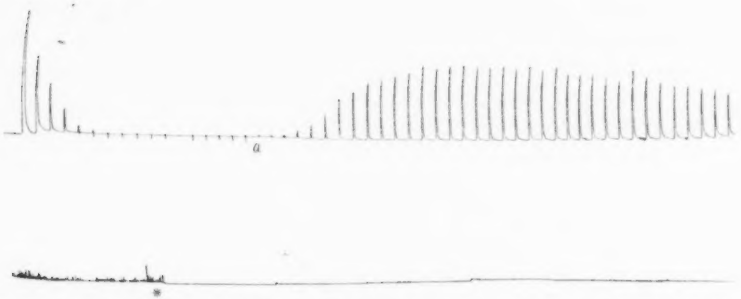


FIGURE 2. — Upper curve. — The sartorius muscle of the frog alternately stimulated by tetanic stimuli of fifteen seconds' duration and then immersed into 0.7 per cent NaCl for forty-five seconds. At (*a*) 0.072 per cent $CaCl_2$ added to the saline. Lower curve. — Sartorius muscle of frog immersed in 0.7 per cent NaCl, but not stimulated. At * 0.072 per cent $CaCl_2$ added to the saline.

This apparent discrepancy finds an explanation in the assumption once more of two contractile elements in the muscle. One of the elements on this supposition was found to be very sensitive to potassium chloride; the other just the reverse, so that it reached a maximum contraction only in twenty-four hours. Calcium chloride as a 1 per cent solution, on the other hand, was found to diminish the contractions of the first contractile element, and its action on the second was much more rapid than that of potassium, producing a maximum contraction in one and one-half hours. If sodium occupies a position between potassium and calcium in its ability to produce its effect upon the two contractile elements, then the salts in the order of their efficiency upon the first element would stand potassium, sodium, calcium. In their efficiency of producing an effect upon the second element they would stand in the reverse order, — calcium, sodium, potassium. If the twitches of the sartorius are due to the action of

the sodium salt on the first contractile element, then the addition of calcium would prevent them, since calcium inhibits the activity of this element. After the preliminary potassium-contraction potassium inhibits the activity of muscle tissue so that the addition of this salt would also prevent the twitches. Weakened contractions resulting from periodical electrical stimulation in saline and involving both contractile elements might be augmented by the addition of calcium, but since one element is inhibited and the other favored, variations in the extent of the recovery might be expected, and this in fact may be noticed. In some cases the recovery may be considerable, as in Fig. 2, while in other cases the effect produced is hardly more than a retardation of the decrease in height of the individual contractions.

To the two hypothetical contractile elements of the sartorius can be added a third, present in the ventricular muscle of the terrapin heart, which manifests a still greater sluggishness to the action of potassium chloride, responds with more vigorous contractions to immersion into sodium chloride, and is by far more affected by calcium chloride. In such a scheme the second contractile element of striated muscle is more nearly similar in its reactions to cardiac muscle than is the first, but the differences and similarities between the three are not absolute, but rather a matter of degree. Dr. Howell has called the author's attention to the difference between the behavior of terrapin's striated muscle and cardiac muscle when subjected to chloroform vapor of a certain degree of density. The striated muscle will contract vigorously, but the heart muscle relaxes with almost equal rapidity. Nevertheless, if the chloroform is made to act with greater rapidity and density, both kinds of tissue shorten.

With regard to the action of the ions of potassium, sodium, and calcium, the idea of the differences between skeletal and cardiac tissue being one of degree can be maintained on the assumption that the first and second contractile elements of the sartorius and the contractile element of the heart form a series of different sensitiveness to the ions in question. Thus the first contractile element of the sartorius is readily affected by the potassium ions, the second contractile element less so, and that of heart tissue not at all unless subjected to special treatment such as previous immersion into cane-sugar. Again, under the influence of sodium chloride the first contractile element responds by twitching, the second element possibly gives rise to larger and less numerous contractions, and heart tissue responds with well-developed co-ordinated contractions. That the second contract-

ile element of the sartorius may respond to immersion into sodium chloride is suggested not only by experimental results, but by the statements of Biedermann and Loeb,[1] who have described an alternation of minute twitches and larger contractions when striated muscle is placed in sodium solutions. Zenneck[2] has described the behavior of the curarized sartorius in $\frac{n}{10}$ sodium bromide solutions as consisting of twitches grouped together so as to appear like blood-pressure curves showing respiratory waves. Lastly, the first contractile element of the sartorius is not at all or very little stimulated by calcium; the second contractile element more so, and heart tissue most of all.

It is impossible to state to what the differences in the reactions of the three contractile substances towards the chlorides of potassium, sodium, and calcium are due. But it is suggested by the experiments with pure solutions that the ion content of the muscle is of great importance. The power of potassium to produce a shortening is impossible when only potassium and sodium are present in the muscle. A physiological percentage of calcium favors the potassium contraction, but when the amount is greatly increased it markedly retards the same reaction. Calcium when applied to a tissue in which the ions are present in physiological amounts leads generally to a shortening, but when applied at the height of a potassium contraction leads to rapid relaxation. Considerations of this kind are also of service in forming a conception of the reasons underlying the less constant variations which muscle exhibits.

As early as 1887 Ringer[3] and Ringer and Buxton[4] undertook a comparison of the effects of sodium, calcium, and potassium solutions upon skeletal and cardiac muscle. They did indeed find both differences and similarities, but certain conclusions in the two papers seem radically opposed. These are attributed to differences in the method of application of the solutions, — immersion in one case and perfusion in the other. Thus upon immersion and periodical stimulation of the sartorius Ringer found that skeletal muscle differed from cardiac muscle in the following particulars: "Contractility is not improved or restored by adding lime salts to saline." "The contractions in saline of a muscle previously weakened by frequent contrac-

[1] Loeb: Ueber Ionen welche rhythmische Zuckungen der Skelettmuskeln bevorrufen, Festschrift für A. Fick, Braunschweig, 1899.

[2] Zenneck: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 21.

[3] Ringer: Journal of physiology, 1887, viii, p. 20.

[4] Ringer and Buxton: Journal of physiology, 1887, viii, p. 288.

tions are strengthened by adding to saline a potash salt." In the later paper by Ringer and Buxton the experiments were performed by perfusing the gastrocnemius with a 0.6 per cent saline solution and stimulating periodically. It was found that excitability had about ceased in an hour and three-quarters. "Upon replacing saline by phosphate of calcium saline a return of contractility took place." "We then returned to saline, and again contractility disappeared, to be restored by returning to the phosphate of calcium saline; and this manœuvre was effected several times, always with the same results." In the experiments underlying this paper in which the frog's sartorius was simply immersed in the solutions and stimulated periodically, potassium was never observed to favor contraction, — its effects being rather the reverse. Calcium, however, after sodium chloride exhaustion almost invariably increased the height of the contractions to some extent, although as stated by Ringer the extent to which recovery occurs varies greatly in different experiments. The experiments in Ringer's earlier paper were performed during the months of May and June, when frog preparations are liable to manifest abnormalities of behavior.

As far as direct experimental evidence goes it would seem that the sartorius is characterized by responding with a rapid contraction when submitted to a 1 per cent solution of potassium chloride, — a result which does not occur in heart tissue when in a fresh condition, — and that this forms a point of difference between the two tissues. Another difference is the reaction to physiological amounts of calcium chloride after treatment with 0.7 per cent saline. Calcium under these conditions inhibits the activity of the sartorius while it augments the activity of heart tissue. But these dissimilarities cannot be looked upon as the expression of sharply marked differences of structure of the two tissues, since treatment with cane-sugar makes ventricular tissue responsive to potassium, and periodic stimulation of the sartorius while in saline changes the inhibitory effect of calcium to an augmentor effect. The latter phenomenon finds an explanation in much better accordance with other known facts by the assumption of two contractile elements in the sartorius, which are differently affected by calcium. On this basis the fibrillary twitches of the sartorius and the rhythmical beats of heart tissue would not be strictly analogous, since they are, to a large extent at least, the manifestations of contractile substances with different properties.

Conclusions.

1. That a supply of the ions sodium, calcium, and potassium in definite proportions is necessary to the best maintenance of the normal activity of skeletal muscle of the frog and heart muscle of the terrapin.

2. That in physiological proportions sodium and potassium produce a condition of relaxation, while calcium produces a condition tending toward contraction, provided all three ions are present in the medium which bathes the muscle. This is true for both heart and skeletal muscle.

3. That the effects of the three ions as stated in 2 are not necessarily the same when the ions are supplied separately to fresh tissue. In isotonic solutions sodium produces a relaxation in both heart and skeletal muscle. Calcium produces a slow contraction in both. Potassium produces two contractions and relaxations in the sartorius, but only relaxation in a fresh strip of the terrapin's ventricle.

4. That the condition of the tissue influences the effect produced by any one ion. Thus previous treatment with sodium chloride, calcium chloride, potassium chloride, or cane-sugar solutions alters the condition of the tissue so that its reactions are modified.

5. That the effect of any one ion may be modified by the simultaneous application of other ions.

6. That the rapid contraction in skeletal muscle produced by a 1 per cent potassium solution and the slow contraction resulting from immersion into a calcium solution are restricted to the portions immersed.

7. That the sodium twitches in skeletal muscle and the rhythmical contractions in heart muscle are at first limited to the immersed parts, but soon spread to those portions not in the solution.

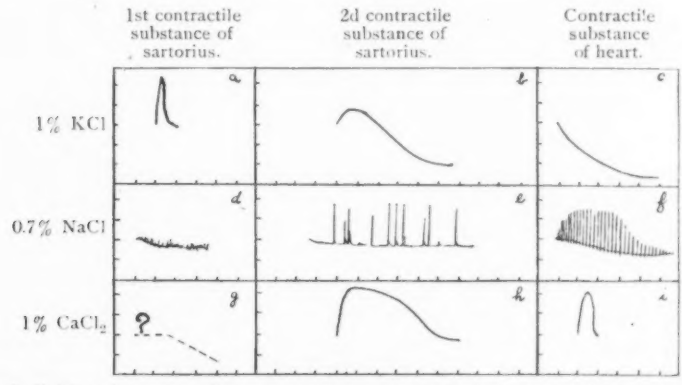
8. That the contraction and relaxation produced by a potassium solution when applied to the sartorius will not occur unless both sodium and calcium are present in the tissue.

9. That the power of potassium chloride to produce a contraction is antagonized by the presence of either sodium or calcium in the bathing medium, but the antagonism of calcium is the more marked.

10. That the effects produced by an alternation of potassium and sodium, on the one hand, and of potassium and calcium, on the other, lend support to the view that the calcium ion is more intimately associated with the contraction process than is the sodium ion.

11. The assumption is made that there are two contractile substances in the sartorius muscle and a third in the terrapin's ventricle. No suggestion is made as to the relation of these substances to definite histological elements, as in the views proposed by Grützner and by Bottazzi. Evidence, however, is offered that the contractile substance of the heart in its properties bears a closer resemblance to the second than to the first contractile substance of skeletal muscle. These substances differ in their sensitiveness to the action of sodium, potassium, and calcium chloride. The first contractile substance of the sartorius responds quickly with a contraction when subjected to a 1 per cent solution of potassium chloride; the second contractile element responds much more sluggishly, and ventricular muscle when in a fresh state gives only relaxation. Calcium chloride in a 1 per cent solution produces no contraction of the first contractile element of the sartorius, gives rise to a slow contraction of the second contractile element, and produces quite a vigorous contraction of heart muscle. Immersion into a 0.7 per cent solution of sodium chloride is followed by fibrillary twitches of the first contractile element of the sartorius; by larger but infrequent contractions of the second contractile element, and by well-developed rhythmical beats in heart strips.

The following diagrams may serve to illustrate these differences. No significance can be attached to the exact form of the curves, but the time relations in a general way are reproduced.



Each division along the ordinates represents a change in the length of the muscle of six millimetres. Each division along the abscissæ is equal in (*a*) to one minute; in (*b*) to one day; in (*c*) to one day; in (*d*), (*e*), and (*f*) to fifteen minutes; in (*h*) to one day; and in (*i*) to one hour. (*g*) represents a supposed relaxation of the first contractile substance in 1 per cent $CaCl_2$.

This assumption offers an explanation of the different effects of small amounts of calcium upon the fibrillary twitches of the sartorius on the one hand, and upon the contractions of the muscle when periodically stimulated, on the other hand. Calcium stops the twitches because it inhibits the activity of the first contractile element which produces the twitches. The contractions of the sartorius when stimulated electrically involve the second contractile substance as well as the first, and calcium in suitable amounts favors the activity of this element. Upon this assumption, furthermore, the fibrillary twitches of the sartorius in sodium chloride are not strictly analogous to the beats of heart tissue in sodium chloride, since they involve the activity of different substances.

THE EFFECT OF CERTAIN SALTS AND DEXTROSE ON THE RATE OF TRANSFORMATION OF GLYCOGEN INTO DEXTROSE.

By C. HUGH NEILSON and OLIVER P. TERRY.

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CLAUDE BERNARD[1] showed that the excess dextrose of the blood is stored in the muscles and liver in the form of glycogen, and that after death the glycogen rapidly disappears from the liver substance. He assumed that during life the glycogen is easily transformed into dextrose, in quantities sufficient for the needs of the animal.

Chemistry shows that equilibrium plays an important part in all chemical actions and enzymatic action. Therefore it is probable that equilibrium is an important factor in the storing up of glycogen, and also in the reverse process, the forming of dextrose from the stored glycogen. That is, when the dextrose reaches a percentage higher than that found normally in the blood, it is stored as glycogen, and when it becomes lower in amount than that found normally in the blood, the glycogen is broken down into dextrose. It is probable that one and the same enzyme is responsible for this double action.

It has been shown by many observers that neutral salts may either stimulate or retard enzyme action. That is, stimulation occurs when the anion is more powerful, and retardation when the kation is more powerful.

The object of this paper is to determine, first, whether increasing the dextrose retards the decomposition of the glycogen in the liver; second, whether calcium chloride, which has a retarding action, and sodium citrate, which has an accelerating action, on enzymes in general, have the same effect on the enzyme which transforms glycogen into dextrose.

[1] CLAUDE BERNARD: Comptes rendus, 1877, lxxxv, p. 579.

Very little work has been done to show the effect of substances on the rate of transformation of the glycogen into sugar. But Cavazzani[1] found that a dog poisoned with quinine had less sugar in the liver than those not poisoned; evidently the quinine retarded the decomposition of the glycogen.

Fränkel[2] found that trichlor acetic acid hindered the formation of sugar from the glycogen in the liver.

Demant[3] showed that phenol hinders the decomposition of glycogen in the liver and muscles, after death.

Methods.

The livers of dogs and rabbits were used in these experiments. The glycogen was isolated according to Pflüger's[4] method. One hundred grams of liver substance were thoroughly ground and mixed, one hundred cubic centimetres of boiling 60 per cent potassium hydrate added, and then this mixture placed on a water bath for two or three hours. This was then cooled, and mixed with two hundred cubic centimetres of water and four hundred cubic centimetres of 96 per cent alcohol. After standing, this was filtered, and the precipitate washed once with one volume of 15 per cent potassium hydrate plus two volumes of 66 per cent alcohol, and once with 66 per cent alcohol. The precipitate was dissolved in hot water and neutralized with hydrochloric acid. This was again filtered, resulting in a clear, opalescent filtrate. The glycogen was inverted by adding hydrochloric acid up to two and two-tenths per cent of its volume, and then it was placed on a water bath for two or three hours. The resulting sugar was then determined by the polarimeter and by quantitative reduction methods. In determining the amount of dextrose present in the resulting mixture, we used Haines' quantitative method for the determination of sugar, checking these results by the polarimeter. The Haines' method was used in preference to Fehling's method, it being just as accurate but more easy of manipulation.

Haines' quantitative solution consists of the following:

Dissolve 8.314 grams of pure copper sulphate in 400 c.c. of distilled water. Add 40 c.c. of pure glycerin. Then add 500 c.c. of a 5 per cent solu-

[1] Cavazzani: Archives italiennes de biologie, 1899, xxxii, p. 350.
[2] Fränkel: Zeitschrift für physiologische Chemie, 1892, lii, p. 125.
[3] Demant: Zeitschrift für physiologische Chemie, 1879, iii, p. 200.
[4] Pflüger: Archiv für die gesammte Physiologie, 1902, xciii, p. 163.

tion of potassium hydrate. Mix thoroughly and make up to one litre with distilled water.

10 c.c. of this solution are reduced and decolorized by 0.01 gram of dextrose.

In carrying out the determination of the dextrose, the method is as follows:

10 c.c. of Haines' solution were placed in a 250 c.c. Erlenmeyer flask, together with 30 c.c. of strong ammonia, — (this was added to produce an atmosphere of ammonia, and thus the oxidation of the cuprous oxide is avoided). This mixture was brought to gentle boiling and the saccharine solution run in slowly from a burette until the blue color just disappeared. The results are uniformly constant when due care is taken in the manipulation.

When, by the preliminary titration, the saccharine solution was found to be strongly saccharine, several dilutions were made, as the end point is not so sharp in dilute solutions as with the concentrated solutions. This method, in our hands, gave equally as good results as the Fehling method. These results were checked by the polarimeter.

The results obtained are shown in the following experiments:

A. Experiments with Ground Liver.

Experiment 1. — To show the effect of adding dextrose, sodium citrate, or calcium chloride, on the rate of transformation of glycogen into dextrose, determining the amount of glycogen in terms of dextrose.

A dog was killed by bleeding and the liver taken out at once. The lobes were cut off, leaving as a residue, the large blood-vessels, gall-bladder, and supporting tissue. The liver substance was then ground in a meat chopper and thoroughly mixed. Five lots of this pulp, of fifty grams each, were then treated as follows:

(*a*) To the first lot, 50 c.c. distilled water were added and the mixture boiled. This was to serve as a control for the amount of glycogen then present.

(*b*) To the second, 50 c.c. distilled water were added. This was to show the normal rate of transformation of the glycogen into dextrose.

(*c*) To the third, 50 c.c. of $\frac{m}{16}$ sodium citrate in $\frac{m}{8}$ sodium chloride were added. This was to show the action of the sodium citrate.

(*d*) To the fourth, 50 c.c. $\frac{m}{16}$ sodium citrate in $\frac{m}{8}$ sodium chloride, plus one gram of dextrose, were added. This was to show the effect of increasing the dextrose on the rate of transformation.

(*e*) To the fifth, 50 c.c. of $\frac{m}{16}$ calcium chloride in $\frac{m}{8}$ sodium chloride were added. This was to show the effect of the calcium chloride.

These mixtures were then placed in an incubator registering 39.5° C. and left for four hours. At the end of this period the glycogen in each was determined according to the method described above. After inverting, the dextrose, which is a measure of the glycogen found, was determined by the Haines quantitative method, and checked by the polarimeter.

(*a*) gave 0.51 gram of dextrose.
(*b*) " 0.264 " " "
(*c*) " 0.193 " " "
(*d*) " 0.454 " " "
(*e*) " 0.316 " " "

Experiment 2.— Two rabbits were killed by bleeding. The livers were ground, mixed, and treated as in experiment 1. Five lots of fifty grams each of liver pulp were also used in this experiment.

(*a*) gave 0.589 gram of dextrose.
(*b*) " 0.266 " " "
(*c*) " 0.179 " " "
(*d*) " 0.415 " " "
(*e*) " 0.368 " " "

By consulting experiments (1) and (2), it is seen: that calcium chloride has a retarding action on the decomposition of the glycogen into dextrose; that sodium citrate has a stimulating action on the rate of transformation; and that increasing the amount of dextrose also retards the rate of transformation. This latter fact well illustrates that in chemical and enzymatic reactions the heaping up of the products of the decomposition retards the rate of the decomposition.

B. Perfusion Experiments.

Experiment 3. This experiment is to show the effect on the rate of transformation of glycogen into dextrose, by perfusing calcium chloride through the liver ($\frac{m}{8}$ sodium chloride was used as a control, instead of distilled water).

In these experiments, instead of grinding the liver and adding the solutions to this pulp, the liver was perfused with the solutions to be tested. Canulæ were inserted into the branches of the portal vein, through which the solutions were passed. The outlet was through a canula inserted in the vena cava inferior.

The animal was killed by bleeding, and with the liver *in situ*, the canulæ were inserted into the vessels. The vessels of one lobe were ligatured, the lobe cut off, ground, some distilled water added, and boiled

at once. This was used to determine the amount of glycogen in the liver at the commencement of perfusion. Through one branch of the portal vein was run $\frac{m}{8}$ sodium chloride; and through a second branch was run $\frac{m}{16}$ calcium chloride in $\frac{m}{8}$ sodium chloride. The liver with the connecting canulæ was placed in a large dish containing $\frac{m}{8}$ sodium chloride and kept at a temperature of 40° C. The solutions were kept at 40° C. and run in under moderate pressure. They were run in at short intervals, the time of perfusion being two hours and the amount of solution 1000 c.c. At the end of the perfusion, the liver was ground and the excess fluid decanted off. The glycogen was isolated and the sugar inverted and determined according to the methods heretofore described.

Objection might be made to perfusing two solutions through different lobes at the same time, as the perfusion might be more rapid and the cells brought into more intimate contact with the solutions, in one lobe than in the other. However, as the results were constant in every experiment, and as the solutions perfused with equal ease in each lobe, this objection is unwarranted.

The results obtained in this experiment are as follows:

100 grams of the boiled liver gave 0.68 gram of dextrose.

100 grams perfused with $\frac{m}{16}$ calcium chloride in $\frac{m}{8}$ sodium chloride gave 0.455 gram of dextrose.

100 grams perfused with $\frac{m}{8}$ sodium chloride gave 0.301 gram of dextrose.

This experiment shows that the amount of glycogen not transformed into dextrose is much greater in the mixture of calcium chloride and sodium chloride than in the sodium chloride alone. That is, the calcium chloride inhibited the rate of transformation.

Experiment 4. This experiment is to determine the effect of sodium citrate and of dextrose on the rate of transformation of glycogen into dextrose.

The method was the same as that used in experiment 3, except that in this case three canulæ were inserted into branches of the portal vein. The vessels of one small lobe were ligatured, the lobe cut off, ground, and boiled.

Into the liver by one canula, 800 c.c. of $\frac{m}{8}$ sodium chloride solution were run. Into a second canula, 800 c.c. of $\frac{m}{16}$ sodium citrate in $\frac{m}{8}$ sodium chloride, were run. Into a third canula, 800 c.c. of $\frac{m}{16}$ sodium citrate in $\frac{m}{8}$ sodium chloride plus sixteen grams of dextrose, were run.

These solutions were run in at short intervals, the 800 c.c. of the different solutions being passed through the liver in two hours. The individual lobes were cut off, ground, and, together with the control, treated as mentioned in the previous experiments.

50 grams of the boiled liver gave 0.57 gram of dextrose.

50 grams of the liver perfused with $\frac{m}{8}$ sodium chloride gave 0.265 gram of dextrose.

50 grams of the liver perfused with sodium chloride plus sodium citrate gave 0.163 gram of dextrose.

50 grams of the liver perfused with $\frac{m}{16}$ sodium citrate in sodium chloride plus 16 grams of dextrose gave 0.325 gram of dextrose.

In this experiment the boiled liver gave the amount of glycogen at the beginning of perfusion. The $\frac{m}{8}$ sodium chloride was used as a control from which to determine the effect of the dextrose and the citrate.

From this experiment it is seen: that sodium citrate has a stimulating action; that increasing the dextrose decreases the rate of transformation of the glycogen into dextrose.

This experiment was also carried out by using blood diluted once with $\frac{m}{8}$ sodium chloride, instead of using pure sodium chloride as the standard. The results confirm those in which the sodium chloride was used. These results were also checked by the polarimeter.

Experiment 5. A few experiments were done to determine the effect on the rate of transformation of adding extract of pancreas and extract of muscle to the solutions used. These results showed: that pancreas extract added to sodium citrate yields more dextrose than when sodium citrate alone is used; that muscle extract has no such effect.

More experiments are being done to determine this more accurately; they will soon be reported.

Conclusions.

It is seen by these experiments that by increasing the dextrose, which is a product of the decomposition of glycogen, the rate of this decomposition is retarded. This agrees with the facts that the products of a chemical reaction retard that action, and that when the blood is rich in sugar, there is a decreased transformation of the glycogen into dextrose.

It is also seen that calcium chloride has a retarding action, and sodium citrate an accelerating action, on the breaking up of glycogen. This agrees with the fact that such salts as sodium citrate, acetate, etc. are used as therapeutic agents to increase elimination, probably by stimulating the cell substance and its enzymes, possibly by making the cell membranes more permeable.

It also agrees with the fact that calcium chloride has a retarding action in elimination, especially in salivary secretion and inhibiting

the secretion of sugar in phloridzin diabetes, as was shown by O. H. Brown and Martin H. Fisher, working independently. This latter fact may possibly be explained by the calcium chloride lessening the permeability of the kidney membranes; but possibly by retarding or inhibiting the transformation of the liver glycogen and the muscle glycogen into dextrose.

Further work is being done by us to determine this latter point.

THE NATURE OF THE PROPAGATION OF NERVE IMPULSE.

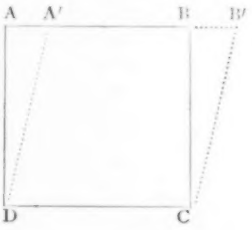
By WILLIAM SUTHERLAND.

THE electrical properties of nerve have received so much attention that it is necessary to preface the following seemingly purely mechanical theory of the propagation of nerve excitation with a few words of explanation. I have recently, in the "Philosophical Magazine," shown that cohesion and rigidity are essentially electrical properties of matter; so that in the tracing of the "conductivity" of nerve to the rigidity of its substance, as I propose to do, the electric properties of nerve are not ignored, but their principal effect is conceived in the simpler and more direct terms of mechanics instead of the ultimate electrical ones. A complete electrical theory of elasticity will have to account for the piezoelectricity of quartz, as well as for the electrical properties of nerve.

Two lines of thought lead to a conception of the possible importance of rigidity in the phenomena of nerve and muscle. In the first place it is known that a jelly offers but little more resistance to the passage of a small ion than does pure water at the same temperature, despite the enormous difference in the large scale viscosities of the two media. This proves that in a jelly the molecules of the gelatine form a mesh dividing the jelly into compartments with net-work walls which confine the molecules of water in batches. The cellular structure gives to the jelly its rigidity, yet the meshes are so open that an ion urged forward by electric force has little difficulty in passing from one compartment to another, and encounters most of the resistance to its motion in passing through the batches of water molecules. Thus the jelly has rigidity on the molar scale, and fluidity on the molecular. Now the rigidity of the jelly is the outcome of the elastic properties of the fibres forming the meshes. Just as an ion moves through the jelly almost independently of the presence of the network, there ought to be phenomena of the jelly confined to the network as regards cause and effect. How would it be possible to

propagate disturbance through a jelly without appreciably affecting its contained water, as a diver signals by his rope to the man in charge of the air pump? It seemed to me that muscular contraction and nerve conductivity might be physiological answers to this query.

The second line of thought was this, that the slowness of the propagation of nerve impulse is such as would probably connect it with the small rigidity of the soft tissues in the animal body. Considering the enormous advantage to an animal of possessing a high speed of nervous signalling, one is impressed by the extraordinary smallness of the actual speed. The following list of velocities brings out this point. In centimetres per second: light, 3×10^{10}; sound in water, 1.4×10^{5}; sound in air, 3.3×10^{4}; nerve excitation, 3×10^{3} (Helmholtz).



In spite of its definite electrical properties, nerve does not transmit excitation by direct electromagnetic vibrations along its substance, like light or electromagnetic waves propagated along a wire. Again, sound is transmitted through the soft parts of the body (treated as water) fifty times as fast as the nerve impulse.

The next step in this line of inquiry is to estimate the speed of propagation of shearing strain through soft tissues, due to their rigidity physically defined in the following way. Let $ABCD$ be the section of a unit cube which has the face represented by CD fixed, while a tractive force T is applied to the unit face represented by AB, displacing AB to the position $A'B'$. Suppose the distance AA' to be small, and the angle ADA' denoted by θ; then Hooke's law for the case of rigidity gives the relation $T = n\theta$, in which n is the rigidity of the substance. By the physical theory of elasticity, the velocity with which a shear like the strain just considered is propagated through the substance is given by the formula

$$V = \sqrt{\frac{n}{\rho}} \qquad (1)$$

where ρ is the density.

I do not know of any direct determinations that have been made of n for the tissues of the animal body; but Young's modulus for such tissues was measured by the physicist Wertheim in 1847 (Annales de Chimie et de Physique, xxi) in the case of human bone, tendon,

muscle, artery, and vein. Young's modulus depends on both the rigidity and the bulk modulus (reciprocal of the compressibility). For a substance which is elastically homogeneous and isotropic, the Young's modulus q is connected with rigidity and bulk modulus k by the relation

$$\frac{1}{q} = \frac{1}{3n} + \frac{1}{9k} \qquad (2)$$

Now muscle and nerve and artery are not isotropic, their structure is different longitudinally from laterally; but as they are mostly water, k has a large value compared to that of either q or n in any direction. So in equation (2), we may put $1/9k = 0$, and so get $q = 3n$ for the soft tissues. Hence for these we can find an average rigidity by taking one-third the value of Young's modulus, and we can calculate the desired velocity V by means of (1) with Wertheim's values of q and ρ. Until recently, Wertheim's values were summarized in text-books of physiology. In the "General Physiology" of M'Kendrick (1888), they are given with a change of unit in which they have been made ten times too large. Some of them are given in a "Text-book of Human Physiology" by Landois and Stirling (1885), along with values by Wundt, though it is not made clear which are Wertheim's and which Wundt's. Accordingly, the following information about Wertheim's determinations is taken from his original paper. His measurements on the tissues were made three or four days after death. To find whether this interval of time made much difference, he compared the results obtained from the tissues of a Newfoundland dog immediately after death, and five days after, finding good enough agreement in the results. For muscle, he found q in kilograms weight per square millimetre to range from 1.271 for a child one year old to 0.261 for a man of seventy-four. The mean of four such samples is 0.68. To convert this number to C. G. S. units, that is, to dynes per cm.^{2}, we multiply by 980×10^6 and get for average human muscle $q = 67 \times 10^6$ dynes per cm.^{2}. Its average density is 1.06, and hence

$$V = \sqrt{\frac{q}{3\rho}} = 4700 \text{ cm. per sec.}$$

For passive muscle, Wundt (Landois and Stirling) gives $q = 0.27$ kgm. per mm.^{2}, whence $V = 2900$ cm. per sec.

For q in the case of nerves, Wertheim finds for four human sciatic nerves, the values 10, 24, 14, and 14, the mean being 15.5, which in

C. G. S. units becomes 1520×10^6. The average ρ is 1.035, whence $V = 22000$ cm. per sec. According to a value which seems to be Wundt's (Landois and Stirling) for nerve $\eta = 1.09$ kgm. per mm.^{2}, which is 107×10^6 C. G. S. Thus the velocity V, with this datum, becomes 5900 cm. per sec.

For actually measured velocities of the nerve impulse, we have Helmholtz's classical determination of 3000 cm. per sec. in the human arm. Waller makes this from 5000 to 6000, quoting Alcock's value 6600 as perhaps better (Nature, Dec. 17, 1903). Gotch, in Schäfer's "Text book of physiology" gives 2700. Bernstein gives 3000 for the rate of propagation of the electric negative variation in nerve. Charpentier finds this rate 2600 in the case of the frog (Comptes rendus, 1901, 132). The highest of these velocities, namely, 6600 lies between the two values calculated above for nerve, namely, 5900 from Wundt's datum, and 22000 from Wertheim's. It has been proved then that the velocity of the nerve impulse is of the same order of magnitude as the velocity of propagation of a shearing stress along a dead nerve. In addition to the observed nerve velocities given above, ranging from 6600 downwards, there are velocities as low as 800 measured in electrical fish, 400 in octopus, 1 in anodon, and 0.1 in eledon (Schäfer's Text book). It is generally accepted that reduction of temperature lowers the nerve velocity considerably, that of a frog falling from about 2700 at 15° C. to 100 at 0°. But Weiss, by cooling nerve and muscle separately, claims to have shown (Comptes rendus, 1900, 130), without giving details, that the effect of cooling the nerve alone from 20° to 0° is to produce quite a small variation in the nerve velocity, a reduction of not more than 9 per cent. He ascribes Helmholtz's result to a change in the latent period of muscle, caused by the change of temperature. In general, rigidity increases with falling temperature; but the viscosity of water, which must be the chief retarding factor in propagation of elastic waves in nerve and muscle, increases rapidly with falling temperature. But the viscosity of water being 0.01778 dynes per cm.^{2} at 0°, and 0.010015 at 20°, it is evident that the present theory could not account for the usually accepted great reduction of nerve velocity according to Helmholtz (from 3000 to 100 at these temperatures). But on the present theory we should expect the change to be of the order of magnitude found by Weiss.

Before finishing this quantitative discussion, it may be as well to examine the results for muscle a little more closely. We found

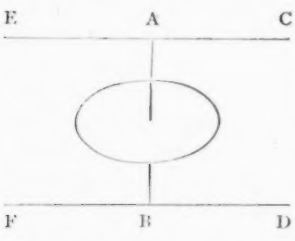
velocities 4700 and 2900 for the propagation of shearing stress in muscle, but the transmission of a muscular contraction takes place rather as the contraction or elongation used in measuring Young's modulus. Hence for a calculated muscle velocity we should multiply these numbers by $\sqrt{3}$, obtaining 8100 and 5000. The observed rates of transmission of contraction in muscle given in Foster's text-book are 300 to 400 for the frog, 500 in the excised muscle of warm-blooded animals, and 1000 in their muscles in the living body. Here the highest observed velocity is only one-fifth or one-eighth of our calculated amount. The reason for this discrepancy is perhaps to be found in time lost during the transmission of the active living pulse from one cell to the next in muscle. The large variation of the latent period of muscle with temperature announced by Weiss would prepare us to expect a phenomenon of the same nature within the muscle itself. But in any case the fact that the calculated and observed velocities are about of the same order of magnitude is the main one at present, and indicates that probably the chief mechanism by which a muscular contraction is propagated is the same as that which gives to dead muscle its elasticity.

As it seemed desirable to add to the above numerical data some direct determinations of the rigidity of artificial preparations similar to the soft tissues, I measured the rigidity of gelatine jelly and coagulated white of egg according to the method described in the experimental introduction to "A Kinetic Theory of Solids" (Philosophical Magazine [5], xxxii), verifying the applicability of the formulæ by varying the length of the specimen within wide limits, and also the moment of inertia of the vibrating mass. For jelly made of 1 part gelatine to 4.2 parts of water, the rigidity at 18° was found to be 2×10^5 dynes per cm.^{2}. Quincke found the elasticity (probably Young's modulus) of glass to be 2×10^6 times that of a 10 per cent jelly, which would correspond to a rigidity of about 1×10^6 dynes per cm.^{2} for the jelly, with which my determination is in reasonable agreement. For hard-boiled white of egg, the rigidity ranged between 9×10^6 for a firm continuous sample to 1×10^6, for one having a granular appearance. Hence the velocity of propagation of a shear in 20 per cent jelly is of the order 400 cm. per second, and in a good sample of coagulated albumen it is of the order 3000. These determinations bear out the contention that the velocity of physiological impulses in soft tissues is of the same order of magnitude as that of physical shears in such tissues.

The next business is to inquire whether any of the phenomena of transmission of the nerve impulse are inconsistent with their being manifestations of elasticity. First: if a nerve is excited at any point in its length, the disturbance is propagated in both directions from the point. This property is also characteristic of an elastic disturbance. Since nerves have special afferent and efferent functions, it is a noteworthy fact that any nerve transmits both afferently and efferently, as it needs must according to the elasticity theory. Second: the blood supply of nerves is so small, and a nerve can conduct impulses for so long without fatigue, that the consumption of energy in a nerve conducting excitation must be decidedly small. In the propagation of vibrations through a rigid body devoid of viscosity, the consumption of energy is nil. Viscosity causes a degradation of the energy of vibration into heat, so that, if a vibration is to be kept up to its original strength, energy would have to be supplied along its course. Possibly in nerve there is a mechanism for making good any small loss of the energy of excitation which may take place along the nerve. The elasticity theory then fits the fact as to the immunity of motor nerves from fatigue. Third: as already pointed out, the elasticity theory cannot account for temperature effects of the order found by Helmholtz, and hitherto accepted by physiologists; but leads us to expect effects of the order determined by Weiss. Further experiment must decide this rather important point. Fourth: recent experiments have demonstrated that some nerve and brain excitations are of an oscillatory nature (Richet and Broca, Comptes rendus, 1897, 124; Charpentier, Comptes rendus, 128, 129, 132, see also his work on the retina). The present theory requires that nervous excitation must be of an oscillatory nature. The frequencies of vibration so far measured for nerves, namely, from 10 to 3000 per second, are much smaller than those to be expected from ordinary electrical vibrations in the nerves. On the elasticity theory, the frequency, being equal to the velocity of propagation divided by the wave length, which is double the length that is thrown into stationary vibration, must depend in the case of nerve on the length which is thrown into stationary nervous vibration. Now at present we do not know whether the periods measured by Charpentier for motor nerves are determined by the external conditions established in his experiments, or by internal conditions characteristic of the nerves studied. I do not know of any structural dimension in nerves that would account for the wave length of 3.5 cm., most frequently encountered by Charpentier in motor nerves of the

frog. On the elasticity theory a predominant wave length would imply some distance of special importance in the building up of a nerve.

Though it is outside of the present thesis to discuss the physical nature of nerve excitation, the following suggestion is brought forward to make quite clear the distinction between the propagation of a mechanical wave in a dead nerve and a physiological one in a living nerve. Though it has just been proposed that excitation is propagated by means of a propagated shear, it is not contemplated to regard the shear as the excitation. I would suggest that the excitation is a molecular motion which can be communicated by a shear. A physical model could easily be made to represent the two separate properties of excitability and conductivity. Take a gyrostat (fly-wheel) rotating round an axis AB, and let the bearings of A and B be attached to wires AC and BD, AE and BF, going to the bearings of other gyrostats. If the gyrostat is in rotation, AB will tend to preserve its direction unaltered. Suppose a blow given to A and to B. This will cause the axis AB to oscillate gyrostatically about its original position. The blow represents a physiological stimulus; the gyrostatic vibration of AB represents the excitation; and the propagation of motion along the wires AC and BD by elasticity represents the conduction of the nerve impulse along a nerve. The rotation of the gyrostat represents the living state of the nerve. If the rotation is stopped, the model represents dead nerve; a blow does not now cause gyrostatic vibrations, but only such as are called forth by the elasticity of the wires. In the same way dead nerve is not excited, but shows mechanical propagation of disturbance travelling with the same order of velocity as is characteristic of the living nerve. The physical mechanism of propagation is the same in both states of the model, just as has been here contended to be the case in the living and dead states of nerve.



As to the particular way in which the shearing strain which propagates a nerve impulse is produced, I think it may be most simply conceived as a torsion in each thread of dry nerve forming the network running through a living nerve like the gelatine framework through a jelly. In measuring the rigidity of a dead nerve we meas-

ure the rigidity of the whole structure built up of framework and included water. In the preceding argument it has been assumed that the rigidity of the whole dead nerve gives a measure of the average effective rigidity of a thread of the network. On this account we must in the first instance look for agreement between our calculated mechanical velocities and the observed physiological velocities only as regards order of magnitude. If the theory is found to be of use to physiologists, it would be interesting to work out mathematically some definite relation between the rigidity of a jelly and the properties of its network.

Since an elastic strain in a body involves electric displacements of the electrons associated with its molecules, it is well to remember that the fundamental distinction between an ordinary electric displacement, such as is produced by the passage of a Hertzian electromagnetic wave through a body, and an elastic electric displacement is this: in the first, the electrons are displaced in such a manner that they cause only slight displacement of their associated molecules, whereas, in the second, the electrons and molecules are subject to the same displacement. In purely electric vibrations the electrons are loaded with only their own inertia; in elastic vibrations they are loaded in addition with the inertia of the atoms. Hence the electric properties of nerve ought to be best understood in the light of a complete electric theory of elasticity, such as will give an account of piezoelectricity and pyroelectricity in the domain of pure physics. In any case there is a splendid field for quantitative work on the pure physics of nerve.

THE EXCRETION OF NITROGEN BY THE WHITE RAT AS AFFECTED BY AGE AND BODY WEIGHT.

By SHINKISHI HATAI.

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THE present work was undertaken for the following reasons: (1) In connection with my work on the effect of starvation on the growth of the nervous system, definite information concerning the normal metabolism became necessary. (2) There are scarcely any records in the literature of the subject in which the metabolism of one animal was investigated according to age (from birth to older age) and weight. The investigations which can be found in the literature along this line are not sufficiently comprehensive to demonstrate the relations existing between the excreted nitrogen and either the body weight or age, since the animals observed were fed with widely different foods and kept under widely different conditions. (3) Exact data concerning the normal metabolism of the white rat are very desirable to have in this laboratory, since a number of our experiments are conducted on this animal.

METHOD.

Experimental cage.— In order to determine the normal nitrogenous metabolism of the rat, it is necessary to collect the urine and fæces. The cage illustrated in Fig. 1 was made especially for this purpose. The construction of the cage is very simple. A cylinder about 21 cm. in diameter is made of fine wire netting. The height of the cylinder is about 18 cm. The base of the cylinder or cage is made of very fine wire netting. The mesh of the bottom wire net must be small enough to hold the fæces or larger food particles. Two holes are made along the wall of the cylinder (*F* and *W*); one hole is connected with the food dish (*F*), and the other with the water dish (*W*). The opening is made square, and one side measures 3 cm. This hole must not be too large, else the animal may pollute the food or water dish by its own excreta. Therefore the size of the entrance must

conform to the size of the animal. The cover of the cylinder or cage is made of wire netting also. The cage thus constructed is placed on a funnel with a diameter of more than 21 cm. Under the tube of the funnel the graduated cylinder (50 c.c.) is placed. The cage and the funnel are held by an ordinary iron stand. Filter paper (*P*) is placed in the funnel so that only liquid can pass into the collector. By keeping the animal in such a cage, the urine alone passes through the filter paper, while the solid particles are stopped either by the filter paper or by the fine wire net which forms the bottom of the cage. The total amount of the urine is directly measured by reading the mark reached in the graduated cylinder. The loss of urine by evaporation is probably very small, for the reason that the surface of the accumulated urine is not exposed directly to the air, but is covered by the filter paper. Even if some evaporation occurred, the relative values would not alter very much, since the room temperature was maintained during all the experiments at between 60° and 70° F. The fæces is removed from the cage by means of a fine, long forceps. The bottom of the cage and the funnel are carefully washed, and the water used is added to the collected urine. From this mixed urine a definite amount (usually 5 c.c.) is measured from the burette for analysis. Nitrogen, both in urine and fæces, was determined by Kjehldahl's method.

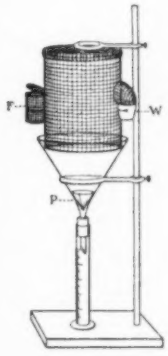

FIGURE 1. — Experimental cage. *F*, food dish; *W*, water dish; *P*, filter paper.

In each case the animal, which was apparently healthy, was kept in the cage for three days, and was fed with "Uneeda Biscuit" alone. Urine and fæces were collected every twenty-four hours, and the nitrogen was determined in each separately. Therefore six determinations were made in each experiment. During an experiment the rats were allowed to eat as much as they desired. At the end of the three days' experiment it was found that the rats under 50 gm. lost slightly in weight; those between 50 and 240 gm. maintained their original weight, while those above 240 gm. gained slightly in weight. This was perhaps due to the fact that the younger animals lacked their habitual active exercise, owing to the small confined space, and lost their appetite, while the older animals, not being so active, were not affected by the smallness of the cage.

One word concerning the use of the "Uneeda Biscuit." Any one familiar with the habits of the rat knows that the animal never likes to leave the food where it is put, but always endeavors to carry it away and hide it under the nest. This habit is not changed while the rat is in the experimental cage. A difficulty arises from this habit, since the rat is almost certain to pollute the food thus removed, and

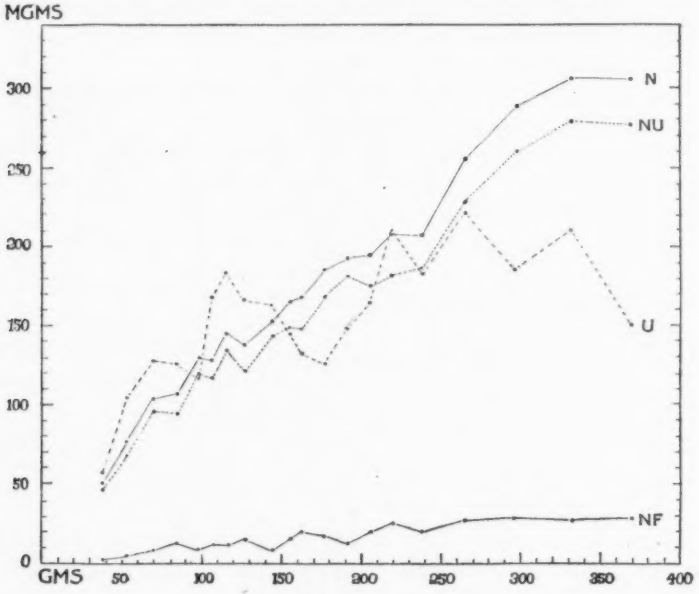

FIGURE 2. — *N*, total nitrogen; *NU*, nitrogen in urine; *U*, urine (c.c.) × 10; *NF*, nitrogen in fæces.

thus the isolation of urine and fæces from the food becomes an extremely difficult task, and the animal may eat such polluted food. To prevent this, "Uneeda Biscuit" which has been soaked in hot water is found very satisfactory, since not more than one bite can be carried away each time, and the animal seems to be discouraged from attempting its removal. Finally, this biscuit contains all the necessary elements of a ration, and does not require any other substance to be mixed with it.

EXPERIMENTAL RESULTS.

The main results obtained during three days' experiments are shown in Table I, as well as by the curves in Fig. 2.

TABLE I.

SHOWING THE AMOUNT OF URINE, FÆCES, AND NITROGEN DURING THREE DAYS. MALE RATS ALONE WERE USED.

Body weight.	No. of animals.	Urine.	Fæces.	Nitrogen in urine.	Nitrogen in faeces.	Total nitrogen.	Body weight.	No. of animals.	Urine.	Fæces.	Nitrogen in urine.	Nitrogen in faeces.	Total nitrogen.
gm.		c.c.	mgm.	mgm.	mgm.	mgm.	gm.		c.c.	mgm.	mgm.	mgm.	mgm.
38	8	5.75	327	52	4	56	162	4	16.13	748	162	32	194
		6.25	217	45	4	49			11.50	208	140	11	151
		5.00	105	42	1	43			12.00	227	141	16	157
Average		5.7	216	46	3	50	Average		13.2	394	148	20	168
53	7	12.62	347	85	11	96	178	4	12.13	379	187	17	204
		9.52	0	65	0	65			12.00	482	154	21	175
		9.17	57	54	3	57			13.38	374	162	15	177
Average		10.4	135	68	5	73	Average		12.5	412	168	18	185
70	8	16.69	395	93	13	106	191	3	16.00	177	194	9	203
		10.87	205	103	7	110			17.30	163	185	9	194
		10.41	68	92	3	95			11.30	348	164	17	181
Average		12.8	223	96	8	104	Average		14.9	229	181	12	193
85	5	15.9	438	97	22	119	207	4	19.30	776	158	29	187
		12.4	219	102	4	106			10.80	516	182	24	206
		9.5	330	83	13	96			19.00	195	181	7	188
Average		12.6	329	94	13	107	Average		16.4	496	174	20	194
99	6	15.50	556	137	20	157	220	2	24.00	809	217	42	259
		10.83	38	124	3	127			20.00	235	181	8	189
		8.98	199	100	5	105			19.00	382	148	29	177
Average		11.8	264	120	9	130	Average		21.00	475	182	26	208
106	6	15.41	374	122	10	132	239	4	18.80	794	207	30	237
		17.67	294	119	5	124			17.80	502	175	15	190
		17.33	248	110	18	128			18.00	404	178	16	194
Average		16.8	305	117	11	128	Average		18.2	566	187	20	207
116	5	22.3	776	143	26	169	266	4	20.38	333	204	21	225
		14.5	138	135	8	143			24.00	896	225	32	257
		18.0	39	123	0	123			22.00	690	259	28	287
Average		18.3	318	134	11	145	Average		22.1	639	229	27	256
127	4	18.25	906	120	26	146	298	5	20.25	956	246	37	283
		13.00	346	115	17	132			18.00	638	272	26	298
		18.75	127	129	6	135			17.35	598	262	24	286
Average		16.7	460	121	16	138	Average		18.5	731	260	29	289
144	5	17.58	359	153	15	168	333	3	16.88	1424	261	25	286
		16.25	360	166	10	176			26.50	475	280	20	300
		15.00	49	113	1	114			19.50	857	297	37	334
Average		16.3	256	144	9	153	Average		20.9	919	279	27	306
156	5	13.90	425	126	14	140	370	3	13.00	877	250	45	295
		13.90	638	151	16	167			12.80	817	289	32	321
		15.75	445	169	17	186			19.30	217	291	9	299
Average		14.5	503	149	16	165	Average		15.0	637	277	29	306

The following statement is based on the averaged figures:

Total amount of urine. — The total amount of urine collected for twenty-four hours varies much more widely than one would expect. The result is shown in Table II.

Generally speaking, from the averaged figures (Table I), the total amount of urine increases with the body weight up to 120 gm., then decreases very decidedly. From 180 gm. it again ascends to 220 gm.,

TABLE II.

SHOWING TOTAL AMOUNT OF URINE COLLECTED DURING TWENTY-FOUR HOURS.

Body weight.	Urine.	No. of animals used.	Body weight.	Urine.	No. of animals used.
gm.	c.c.		gm.	c.c.	
38	5.7	8	162	13.2	4
53	10.4	7	178	12.5	4
70	12.8	8	191	14.9	3
85	12.6	5	207	16.4	4
99	11.8	6	220	21.0	2
106	16.8	6	239	18.2	4
116	18.3	5	266	22.1	4
127	16.7	4	298	18.5	5
144	16.3	5	333	20.9	3
156	14.5	5	370	15.0	3

where it remains rather constant. This is also shown in Curve U. The irregularities which appear between 220 and 270 gm. may not be more than statistical variations, but those between 120 and 220 gm. need consideration. One group urinated a very large quantity at the stage of 100 gm., but the amount became suddenly smaller when the animal reached 120 gm. After passing 180 gm. this group began to urinate again in large quantities. The same fact was noticed in other groups. Therefore a diminution of urine at this period is not a mere statistical variation, but seems to be a normal phenomenon. In addition, the animals at this stage, when the urine is scanty, drink very small quantities of water. Whether or not this is a phenomenon of adolescence needs further investigation. Leaving this peculiar stage of diminution, it is shown in Curve U that the total amount of urine

reaches the end of the rapid rise in the animal of 120 gm.; that is, in the rat about eighty days after birth. Therefore the smaller animal urinates a relatively greater quantity than the larger animal.

By examining the daily excretion of urine during the three days' experimental period, it appeared that the amount of urine in the animals under 100 gm. diminished daily; that in those under 300 gm. it was greatest on the first day, on the next it fell decidedly, and on the last it rose again; while in rats over 300 gm. it increased daily. The

TABLE III.

SHOWING TOTAL NITROGEN EXCRETED DURING TWENTY-FOUR HOURS.

Body weight.	Total nitrogen.	Total urine.	No. of animals used.	Body weight.	Total nitrogen.	Total urine.	No. of animals used.
gm.	mgm.	c.c		gm.	mgm.	c.c.	
38	50	5.7	8	162	168	13.2	4
53	73	10.4	7	178	185	12.5	4
70	104	12.8	8	191	193	14.9	3
85	107	12.6	5	207	194	16.4	4
99	130	11.8	6	220	208	21.0	2
106	128	16.8	6	239	207	18.2	4
116	145	18.3	5	266	256	22.1	4
127	138	16.7	4	298	289	18.5	5
144	153	16.3	5	333	306	20.9	3
156	165	14.5	5	370	306	15.0	3

cause of the different phenomena observed may be explained partially by the fact that the animals under 100 gm. lacked their habitual activities, thus causing a diminution. Under 300 gm. the animals ate a greater amount of biscuit on the first day, — the rat eats a larger amount whenever a new kind of food is given, — but on the next they did not take so much, while in the case of the rats over 300 gm. they were not affected by the altered surroundings and their normal increase in body weight continued.

Total nitrogen. — By total nitrogen is meant the sum of nitrogen found both in urine and fæces. Table III shows the total nitrogen excreted by the rat at different weights during twenty-four hours.

It is clear, from Table III, that the total amount of nitrogen is

quite independent of the amount of urine. The former steadily increases as the animals grow in weight. Unlike the urine, there is no period of rapid increase, but it increases constantly and continuously throughout the portion of the life cycle which is here examined.

TABLE IV.

SHOWING RATIO BETWEEN BODY WEIGHT AND TOTAL NITROGEN EXCRETED DURING TWENTY-FOUR HOURS.

	Body weight.	Body weight. Relative increase.	Total nitrogen.	Total nitrogen. Relative increase.	B.W.:N.		Body weight.	Body weight. Relative increase.	Total nitrogen.	Total nitrogen. Relative increase.	B.W.:N.
	gm.		mgm.				gm.		mgm.		
1	38	1.00	50	1.00	1:.0013	11	162	4.26	168	3.36	1:.0010
2	53	1.39	73	1.46	:.0014	12	178	4.68	185	3.70	:.0010
3	70	1.84	104	2.08	:.0015	13	191	5.02	193	3.86	:.0010
4	85	2.23	107	2.14	:.0013	14	207	5.44	194	3.88	:.0009
5	99	2.60	130	2.60	:.0013	15	220	5.78	208	4.16	:.0009
6	106	2.78	128	2.56	:.0012	16	239	6.28	207	4.14	:.0008
7	116	3.06	145	2.90	:.0013	17	266	7.00	256	5.14	:.0009
8	127	3.33	138	2.76	:.0011	18	298	7.84	289	5.78	:.0009
9	144	3.78	153	3.06	:.0011	19	333	8.76	306	6.14	:.0009
10	156	4.10	165	3.28	:.0011	20	370	9.73	306	6.12	:.0008
	Below 100-gram period (31 rats) . .					..	(1–5) 69	1.	93	1.	1:.0013
	Below 200-gram period (33 rats) . .					..	(6–13) 147	2.13	159	1.70	:.0011
	Above 200-gram period (25 rats) . .					..	(14–20) 276	4.00	252	2.70	:.0009

Therefore, generally speaking, the total amount of nitrogen increases with the weight in such a way as to form an approximately straight line when the total nitrogen is plotted on the base line according to the body weight (see Curve N, Fig. 2). The amount of nitrogen, however, does not increase in direct proportion to the body weight, as the smaller rat excretes a relatively greater quantity than the larger animals. Therefore the curve showing the nitrogen per gram of the body weight forms an approximately straight line which falls gradually

as the animal becomes heavier. This relation between the body weight and the total amount of nitrogen excreted will be more clearly indicated by dividing the latter by the former and comparing the results thus obtained. Table IV shows these results.

Table IV shows clearly that the smaller animal excretes a relatively greater amount of nitrogen. The second and third columns show the relative increase of body weight and nitrogen, and although the largest animal is nearly ten times heavier than the smallest, nevertheless the

TABLE V.

SHOWING TOTAL NITROGEN EXCRETED BY THE RAT AT DIFFERENT AGES.

Age.	Body weight.	Total nitrogen.	No. of animals.	Age.	Body weight.	Total nitrogen.	No. of animals.
day.	gm.	mgm.		day.	gm.	mgm.	
33	45	60	7	85	107	125	4
43	61	92	11	94	153	158	4
55	100	122	6	109	195	220	3
68	99	114	7	Over one year	350	276	Calculated.
78	149	158	7				

nitrogen excreted by the former is only six times greater than the latter. This also means that the body weight and nitrogen do not increase in direct proportion. Thus the nitrogen required per gram of body weight varies according to the size of the animals, and to keep the animal in nitrogenous equilibrium relatively different quantities of food must be used. In the last three lines in Table IV the approximate amount of food required for the nitrogenous equilibrium is shown. For this purpose the entire series of rats is divided into three groups: (1) rats under 100 gm.; (2) rats over 100 and under 200 gm., and (3) rats over 200 gm. Taking the rats under 100 gm. as the standard, it was found that rats between 100 and 200 gm. require 1.7 of nitrogen a day per 100 gm. of rat, and over 200 gm. they require 2.7 times the standard. (See the column next to the last.)

If we arrange the rats according to age, the results thus shown differ from that of Table III in many respects. Table V shows the total amount of nitrogen excreted by the rats at different ages.

Generally speaking (see Table V), the total amount of nitrogen excreted increases as the animals grow older, but here will be found a

distinct period of rapid rise up to 195 gm. in weight, since the animal reaches that stage within a short period, that is, about one hundred and ten days. From this stage on the animal grows continuously but slowly, as it takes more than a year to reach 350 gm. In such an animal (350 gm.) the excreted nitrogen is about 276 mgm., contrasted with 220 mgm. of nitrogen in one hundred and ten days old rats, or an increase made during more than two hundred days is but 56 mgm., or 20 per cent.

Nitrogen of urine and fæces. — As in the case of total nitrogen, that in the urine increases as the animals grow in weight. This is shown in Table VI.

The urinary nitrogen represents nearly 91 per cent of the total nitrogen in the case of smaller animals, and in that of the larger about 89 per cent. Therefore, if the curve of the urinary nitrogen is plotted on the base line according to the body weight, then the character of the curve thus obtained will approximately coincide with that of the total nitrogen. The nitrogen of the fæces increases also according to weight, as is shown in Table VI. The smaller animal eliminates a relatively greater amount of the nitrogen, as in the case of the urinary nitrogen. The percentage distribution of the fæcal nitrogen in the total is about 9 per cent in the case of small, and 11 per cent in the larger animals.

Formulæ for calculating total nitrogen. — From the data given in Table III, it was found that if we plot a curve according to the respective values obtained from logarithm of the total amount of nitrogen and body weight the character of the straight line becomes more prominent. This fact suggested to me at once to draw an ideal line which should pass through as many points as possible. Curiously enough, most of the points fall right on or very near this ideal line. The mathematical value of this line was found to be $x = \frac{233 + 3y}{4}$.

In this formula x = logarithm of the total amount of nitrogen and y = logarithm of the body weight. Therefore the following formula is obtained:

$$\text{Log. } N = \frac{233 + (3 \times \text{log. B. W.})}{4},$$

where N represents nitrogen in milligrams and B. W. represents body weight in grams. The total nitrogen observed and that calculated by this formula are shown in Table VII.

TABLE VI.

SHOWING THE NITROGEN OF URINE (A) AND FÆCES (B) EXCRETED DURING TWENTY-FOUR HOURS.

A.

Body weight.	Total urine.	Nitrogen in urine.	No. of animals.	Body weight.	Total urine.	Nitrogen in urine.	No. of animals.
gm.	c.c.	mgm.		gm.	c.c.	mgm.	
38	5.7	46	8	162	13.2	148	4
53	10.4	68	7	178	12.5	168	4
70	12.8	96	8	191	14.9	181	3
85	12.6	94	5	207	16.4	174	4
99	11.8	120	6	220	21.0	182	2
106	16.8	117	6	239	18.2	187	4
116	18.3	134	5	266	22.1	229	4
127	16.7	121	4	298	18.5	260	5
144	16.3	144	5	330	20.9	279	3
156	14.5	149	5	370	15.0	277	3

B.

Body weight.	Amount of fæces.	Nitrogen in fæces.	No. of animals.	Body weight.	Amount of fæces.	Nitrogen in fæces.	No. of animals.
gm.	mgm.	mgm.		gm.	mgm.	mgm.	
38	216	3	8	162	394	20	4
53	135	5	7	178	412	18	4
70	223	8	8	191	229	12	3
85	329	13	5	207	496	20	4
99	264	9	6	220	475	26	2
106	305	11	6	239	566	20	4
116	318	11	5	266	639	27	4
127	460	16	4	298	731	29	5
144	256	9	5	330	919	27	3
156	503	16	5	370	637	29	3

By comparing the results calculated by this formula with those observed, it is shown, in Table VII, that the difference in averages between the two is extremely small, amounting to +0.004, or less than

TABLE VII.

SHOWING LOGARITHM OF THE TOTAL NITROGEN OBSERVED AND THAT CALCULATED.

Body weight.	Log. N Observed.	Log. N Calculated.	+	−	Body weight.	Log. N Observed.	Log. N Calculated.	+	−
gm. 38	1.70	1.76	0.06	..	gm. 162	2.23	2.24	0.01	..
53	1.86	1.87	0.01	..	178	2.27	2.27	..	..
70	2.01	1.97	..	0.04	191	2.29	2.29	..	..
85	2.03	2.03	..	..	207	2.29	2.32	0.03	..
99	2.11	2.08	..	0.03	220	2.32	2.34	0.02	..
106	2.11	2.11	..	..	239	2.32	2.37	0.05	..
116	2.16	2.12	..	0.04	266	2.41	2.40	..	0.01
127	2.14	2.15	0.01	..	298	2.46	2.43	..	0.03
144	2.18	2.20	0.02	..	333	2.49	2.47	..	0.02
156	2.22	2.22	..	..	370	2.49	2.51	0.02	..
Average						2.204	2.208	0.004	..

+0.2 per cent, in favor of the calculated results. An examination of an individual case reveals still further proof of the accuracy of this formula. The greatest deviation, +0.06, occurred, however, just once out of twenty cases, while in the greater number the frequency of the deviation was ±0.01 and ±0.02. Table VIII shows the relation just mentioned.

TABLE VIII.

Amount of deviation.	±0	±0.01	±0.02	±0.03	±0.04	±0.05	±0.06
Frequency . . .	5	4	4	3	2	1	1

The results obtained by the formula given above are in the form of the logarithmic value of total nitrogen. Therefore, in order to obtain the absolute value of total nitrogen, the logarithmic must be

converted into absolute value. This is done simply by reading the antilogarithm of the respective values, or by the following formula:

$$N = 10^{\frac{233 + (3 \times \log. \text{ B. W.})}{4}}.$$

From the results obtained by this formula we find that in the average the difference is +2 milligrams, or slightly over +1 per cent, in favor of the calculated results. Thus we can calculate the total nitrogen with a high degree of accuracy from the formula when we know the body weight of the animal. It must be remembered, however, that in the present experiment the animals were fed with "Uneeda Biscuit" alone. Therefore any other foods which contain other amounts of nitrogen than the biscuit here used will probably be followed by the elimination of different amounts of nitrogen. Nevertheless, so long as the animals are fed with the same kind of food throughout a given period the main features of the curve should not be altered.

From the above the conclusion is drawn that the total amount of nitrogen eliminated by rats of different weights, fed with "Uneeda Biscuit," may be determined with a high degree of accuracy by the formula here given. Whether or not there exists in other classes of animals a similar relation between the body weight and the total amount of nitrogen excreted cannot be stated without further observation.

Summary.

From observations on eighty-nine male rats at different ages and weights the following results were obtained:

1. The total amount of urine increases with the weight up to 120 gm., then decreases very decidedly. From 180 gm. it again increases up to 220 gm., beyond which weight it remains rather constant. A diminution of urine in animals between 120 and 180 gm., or approximately 70–125 days old, seems to be a normal phenomenon rather than mere statistical variation. Whether or not this is a phenomenon of adolescence needs further investigation. It must be noted, however, that puberty in the rat begins at about seventy days after birth. The smaller animals excrete a relatively greater quantity of urine than the larger animals.

2. The total amount of nitrogen is quite independent of the amount of urine, and increases constantly and continuously throughout life.

It increases with the weight, as is shown in Curve N, Fig. 2. The smaller rats, however, excrete a relatively greater quantity than the larger animals.

3. The percentage value of urinary nitrogen is 91 per cent of the total in the case of smaller animals, and 89 per cent in that of the larger.

4. The total amount of nitrogen eliminated by the rat during twenty-four hours at different weights may be determined with a high degree of accuracy by the following formula:

$$N = 10^{\frac{233 + (3 \times \log. \text{ B. W.})}{4}} \text{ or } \log. N = \frac{233 + (3 \times \log. \text{ B. W.})}{4},$$

where N = total nitrogen in milligrams and B.W. = body weight in grams.

ON THE MASKING OF FAMILIAR IONIC EFFECTS BY ORGANIC SUBSTANCES IN SOLUTIONS.

By PERCY GOLDTHWAIT STILES and WILLIAM HERBERT BEERS.

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IT is the purpose of this paper to call attention to certain facts which seem to have escaped general recognition, though without doubt tacitly accepted by many experimenters. In the numerous researches concerning the effects of ions upon living tissues the solutions used have been usually simple inorganic mixtures, and rarely has any consideration been given to the possible variation of results which might be occasioned by adding proteid or other organic material to the media. Inferences drawn from trials with inorganic solutions have sometimes been carried over without qualification and applied in reasoning about the individual effect of ions in blood and lymph. Great caution should be observed in making such deductions, for the state of affairs in the plasma is far removed from that in a purely mineral solution.

How far this is true may be illustrated by a reference to the alkalinity of serum, much investigated of late. The simple statement used to be made that serum has an alkalinity equivalent to 0.2 per cent sodium carbonate. Every one who has used Ringer's solutions knows that in the absence of proteid such a concentration of sodium carbonate is toxic to tissues and precipitates calcium. Recent study of blood and serum shows that power to neutralize acid is no fair measure of their active alkalinity, for temporary compounds exist between the hydroxyl ions and the proteids. Comparatively few hydroxyl ions are free at any one time. Brandenburg[1] has found that four-fifths of the total alkali is in a form which he feels justified in

[1] BRANDENBURG: Zeitschrift für klinische Medizin, 1902, xlv, p. 157; also FARKAS: Archiv für die gesammte Physiologie, 1903, xcviii, p. 551; FRIEDENTHAL: Zeitschrift für allgemeine Physiologie, 1902, i, p. 56; FRIEDENTHAL: Archiv für Physiologie, 1903, p. 550.

calling indiffusible. Loeb[1] has noted the difficulty experienced in altering the number of free hydroxyl ions in blood by adding acids or alkalies.

The combinations between proteids and acids and alkalies are well known and readily demonstrated by means of freezing-point determinations. The value $\triangle$ is distinctly reduced when proteid is added to an acid or to an alkaline solution the freezing-point of which has been previously ascertained. This method has been used extensively by Bugarszky and Liebermann[2] and by Cohnheim and Krieger.[3] The question as to whether such combinations are always formed when neutral salts and proteids are brought together is not so well settled. Bugarszky and Liebermann did not obtain evidence of such combination between proteid and sodium chloride. On the other hand, Buffa[4] believes that the chlorides of the blood are normally in combination with the proteids. Loeb,[5] Pauli,[6] and others have elaborated the attractive hypothesis that the salts of the tissues and, presumably, those of the body fluids exist largely in such compounds. But the nature of the union is ill-defined. It must be a most slender one, lacking the tenacity to retain the salt when there is a chance for dialysis. Perhaps we may have to recognize *physiological compounds* which are not demonstrable at all by chemical methods but only by the reactions of living tissues. An alternative statement would be that the masking of an ionic effect by a colloid is a most delicate indication of a union between the two.

The hypothesis that ions are arrested or at least restrained in their physiological activity by the colloids of the blood goes far toward explaining the disparity between the sensitiveness of cardiac tissues to changes in the proportions of a Ringer's mixture and the comparative indifference of the heart *in situ* to copious intravenous injections of salt solutions. One of us (S. with R. P. Wadhams[7]) has frequently introduced great volumes of a 1 per cent solution of calcium

[1] Loeb: Studies in general physiology, ii, p. 547 note.

[2] Bugarszky and Liebermann: Archiv für die gesammte Physiologie, 1898, lxxii, p. 51.

[3] Cohnheim and Krieger: Zeitschrift für Biologie, 1900, xl, p. 95.

[4] Buffa: Archives internationales de pharmacodynamie et de thérapie, 1900, vii, p. 425.

[5] Loeb: This journal, 1900, iii, p. 327.

[6] Pauli: Über physikalisch-chemische Methoden und Problem in Medizin, Wien, 1900. Wiener akademische Anzeiger, 1899.

[7] Unpublished work.

chloride into the veins of dogs — sometimes as much as 100 c.c. at once — with only a transient disturbance of the blood-pressure and little or no change in the character of the heart-beat. Making all allowances for rapid exchanges between the blood and the tissues, it remains certain that such infusions must radically raise the average calcium content of the blood and that a shielding action must be attributed to the organic molecules of the medium.

Anticipating that we might find the familiar effects of salts or ions upon contractile tissues lessened or suppressed when organic bodies of large molecule were present in the solution, we chose certain of the best-known cases of ionic influence for investigation. The following suggested themselves as likely to yield clean-cut returns: (1) the heightening of tone by calcium chloride, (2) the more exaggerated and radically toxic effect of barium chloride, (3) the depressing effect of potassium chloride in small amounts, (4) the similar effect of sodium nitrite, the last illustrating the power of the anion. We sought to test the reactions of tissues toward measured concentrations of these four salts in the presence of organic substances, and we made comparisons between the records thus obtained and those of a control series in which no organic materials were added to the media. The organic substances used in the significant experiments included white of egg, partially dialyzed serum, Witte's Peptone and starch. No attempt was made to secure these compounds in ash-free form because their mineral content never amounted to more than a very small fraction of the total salts in solution. The only instance in which the ash had an obvious effect was with white of egg where the depressing power of potassium sometimes showed itself and was offset by modifying the solution to which the egg was added. The contractile tissues tested comprised slips of plain muscle from the alimentary canal of the frog, the terrapin, and in one case the guinea-pig, cardiac preparations from the frog and the terrapin, and also skeletal muscles from the same animals.

The method of experiment was simple. In a small beaker a measured amount of a suitable solution was placed. "Normal" saline or a Ringer's mixture was used as a basis, and the organic body added — if proteid to a concentration of about 2 per cent. In another beaker was an equal quantity of the basal mineral solution. Companion strips of tissue or muscle preparations were suspended in the two beakers, and traced superimposed records of their tone-changes by means of light levers writing upon a very slow drum. Additions

of the salts to be tested were made from burettes, and the concentration of the solutions was kept constant within reasonable limits so as to avoid disturbances of osmotic relations.

The results of about fifty experiments indicate that the effect of a given amount of a salt upon the tone of any of the contractile tissues employed is much obscured by the organic bodies when these are present. To make the response of the preparation in such a solution equal to that of its fellow in a simple inorganic mixture, from two to four times as much of the stimulating or depressing agent must be added.

The effect of barium chloride is one of the most available for study, and in this case skeletal muscle reacts most decisively. The prompt onset of rigor when a minimal quantity of barium chloride is added in the absence of the protecting proteid or starch is in marked contrast to the tardy development of the contraction in the other case. Pauli [1] has shown that barium has the power to combine with proteid, but in very small proportions. Freezing-point determinations of such accuracy as we could attain gave us no evidence of such combination, but there was the best of testimony from the behavior of the muscle.

Of course our experiments are of doubtful value unless one can be assured that the organic bodies employed are themselves nearly or quite without influence upon the tissues. This seemed tolerably certain in most cases. The occasional prominence of the potassium effect with white of egg has been mentioned. Starch does seem to have some influence unfavorable to prolonged activity and sustained tone of the preparations, but it was not such as to obscure the general trend of the results. In the early stages of our experiments we frequently exchanged the solutions that both pieces of tissue might have periods of exposure to the organic substance.

In a few supplementary experiments we tried to find out whether the effect of temperature changes upon the tone of plain muscle is more marked in the absence of organic material than in its presence. There seemed to be no distinct difference; both strips relaxed when warmed and contracted when cooled in the familiar manner and in about the same degree. These negative results are useful inasmuch as they favor the view just now defended that the organic bodies are substantially indifferent to the muscle preparations.

We conclude that the ionic effects so far studied are all masked in

[1] PAULI: *Loc. cit.*

striking manner by organic bodies of large molecule. It would be a difficult and delicate matter to bring this principle to bear in criticism of earlier work. Still the following suggestion may be admissible. Most of the investigators who have studied the relationship between rhythmicity and various conserving media have found it of no advantage to add proteids or other organic bodies to their salt solutions. A few have constantly claimed that some benefit proceeds from such additions. In at least a portion of these cases it seems possible that the only usefulness of the organic ingredient has been to moderate the effect of ions not present in the most desirable proportions and numbers.

The most suggestive result of this work appears to be the corollary to our main conclusion, — that the masking of ionic effects upon living tissues by organic bodies may be taken as an indication of combination where it is difficult to obtain evidence upon the matter by the usual chemical methods.

A MECHANICAL THEORY OF MUSCULAR CONTRACTION AND SOME NEW FACTS SUPPORTING IT.

By EDWARD B. MEIGS.

IN 1897[1] and 1898[2] there were published in the Journal of Anatomy and Physiology two articles which for the first time put forward a comprehensible mechanical theory of muscular contraction. The author deals mainly with the fibrils of the wing muscles of insects, and he advocates the hypothesis that the contraction of these fibrils is caused by their absorbing some of the fluid sarcoplasm which surrounds them. A rough idea of how an increase in the volume of the contents of a fibril might cause it to be shortened can be gained from a consideration of the model shown by Figs. 1 and 2.

In Fig. 1 the model is represented in a state of extension. *A A* is a tube of thin rubber encircled at intervals by wire rings, *E E E*. Along the tube run inelastic cotton threads, *F F F*, each of which is attached to all of the wire rings. The part of the model so far described represents the fibril. *B* is an air-pump, by which air may be forced into the tube, *A A*. When this is done, the model takes the form shown by Fig. 2. Each of the divisions of the tube between the wire rings tends to become spherical, the cotton threads are thrown into curves, and, as they are nearly inelastic, the whole structure necessarily shortens. The illustrations are rough scale drawings of the model itself, so that the relative dimensions are preserved. The distance between the lines *A* and *B*, Fig. 2, represents the amount of the shortening.

A good deal of McDougall's argument is based on the fact that the form of contracted fibrils is somewhat like that of the contracted model. To me his reasoning seems most convincing, and any one who has read his articles must at least admit that a very important question in regard to the physiology of muscle has been opened.

[1] McDOUGALL, W.: Journal of anatomy and physiology, xxxi, p. 410.
[2] McDOUGALL, W.: Journal of anatomy and physiology, xxxii, p. 187.

In April, 1904,[1] I published (unfortunately, without having seen McDougall's papers) an article in which it is shown that the contracted voluntary muscle fibres of the frog are sometimes found in a form strikingly like that of the contracted model. I have now to submit the most direct and positive evidence that during the contraction of this form of muscle fluid passes from the periphery of the fibres toward the centre.

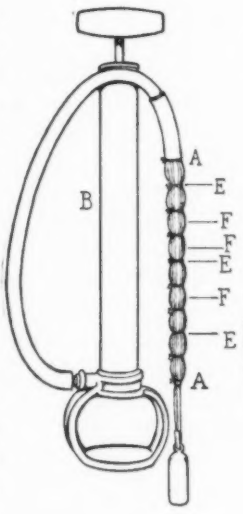


FIG. 1. — Model extended.

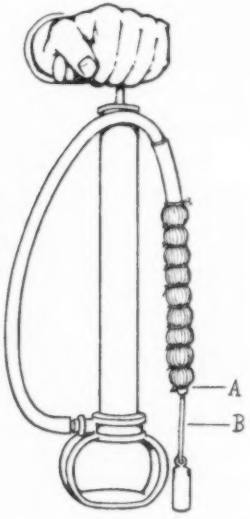


FIG. 2. — Model contracted.

If a frog's muscle be frozen while still living and cut into transverse sections, and if these sections be stained with a 2 per cent methylene-blue solution and immediately examined in 0.7 per cent sodium chloride solution, most of the sections of the fibres will be found in two highly typical, though strikingly different, forms, which are illustrated in Fig. 3. This and the other illustrations are by Hermann Faber. I cannot say too much in praise of the accuracy of the drawing and the skill of the rendering.

A large number of fibres will present the appearance of *A*, Fig. 3. The cross-section of one of these fibres consists of a deeply stained peripheral ring surrounding a faintly stained or unstained central area. The peripheral ring exhibits a more or less plainly marked radial striation; the central area shows a network of dark lines enclosing lighter spaces, which I take to be the fields of Cohnheim. In muscles taken from winter frogs the nuclei are large and abundant; in the case of summer frogs the nuclei are much less prominent or altogether absent. By changing the focus, it can usually be made out that the ring is thicker perpendicularly than the central area;

[1] MEIGS, EDWARD B.: American journal of the medical sciences, April, 1904.

on focussing down the ring comes into focus first and afterwards the central area.

A smaller though considerable number of fibres presents the appearance of *B*, Fig. 3. In these there is a slightly stained or unstained ring, surrounding a deep blue central area. The ring, like that of form *A*, shows radial striations; the central area shows the network of dark lines somewhat obscured by the deep blue color, and in addition a rather granular appearance. It is most important to notice that the relative sizes of the ring and central area have changed. In the *A* form the diameter of the whole section—ring plus central area—is about twice the diameter of the central area alone; in the *B* form the diameter of the whole section is only about half again as great as the diameter of the central area. By changing the focus it can be made out that in the *B* form the central area is thicker perpendicularly than the peripheral ring; on focussing down, the central area comes first into focus and afterward the peripheral ring. In sections prepared by the method described there are numerous other forms less frequently seen, but it will be more profitable to discuss these later. It will now be shown that the fibres presenting the *A* form are relaxed, and that those presenting the *B* form are contracted.

The simplest way of showing this is to compare the cross-sectional appearance of two muscles which have been treated exactly alike in every way, except that one has been frozen while relaxed, and the other while contracted under the influence of a tetanizing current. The experiment may be performed as follows: A cover-glass is laid over the freezing-block to obviate short-circuiting the stimulating current, and a pair of thin copper electrodes over the cover-glass. The freezing-block is then reduced to a very low temperature and the stimulating current started. Interruptions as frequent as one hundred per second seem to give the best results. The muscle is now laid over the electrodes. It can thus be completely frozen in about a minute after the tetanizing current has been applied. The control muscle is put through exactly the same process, except for the application of the tetanizing current. I have gone to the detail of interposing a cover-glass between the freezing-block and the control muscle, lowering the temperature of the freezing-block before laying the muscle on it, preparing the control muscle first in some cases and in others the contracted muscle, etc., in order to get exactly the same conditions in both cases. When the experiment is properly performed, the result invariably is that the sections of the contracted

muscle show more fibres in the *B* form than those of the control muscle. It must be added that by this method not all the fibres of the contracted muscle can be got in the typical *B* form, nor all those of the control muscle in the typical *A* form. The control muscle may almost always be seen to contract slightly when laid on the cold freezing-block and a few of its fibres are usually found in the *B* form. On the other hand, it would be very unlikely that all the fibres could be caught contracted by the above-described method of preparing sections from a muscle in contraction; but the contracted muscle usually shows so many more fibres in the *B* form than the control muscle, that there can be no doubt that the *B* form really is that of contracted fibres.

The experiment which has just been described is quite sufficient to settle the point in question, but still further light is thrown on the subject by the following facts: The number of fibres which appear in the *B* form in sections from muscles frozen while supposedly relaxed depends largely on the irritability of the muscle immediately previous to being frozen. In the case of frogs killed in winter, the great majority of the fibres appear in the *A* form. If the work be done on cool days in summer, nearly as many fibres appear in the *B* form as in the *A* form; and if the work be done on very hot days in summer without the precaution of previously cooling the frog by means of ice, practically all the fibres will be found in the *B* form. Another fact of great interest is that if a muscle be thrown into heat rigor or water rigor, and then frozen and cut into sections, every fibre in every section will be found in a form resembling *B*, Fig. 3.

It may therefore be taken as established that, whatever changes the muscle fibres undergo during the processes of freezing, cutting, and staining, the *A* form stands in the sections as the representative of the uncontracted fibres, the *B* form as the representative of the contracted fibres. It will now be shown that the change from *A* to *B* consists, in all probability, in a passage of fluid from the peripheral ring to the central area.

The most conspicuous difference between *A* and *B* is that the former consists of a darkly stained ring surrounding a faintly stained central area, whereas the latter consists of a darkly stained central area surrounded by a faintly stained or unstained ring. This difference is of itself sufficient to suggest that, as the fibre changes from the *A* state to the *B* state, some fluid which stains with methylene-blue passes from the peripheral ring to the central area. But when

it is added that in the *B* fibres the central area is invariably larger in comparison to the peripheral ring than in the *A* fibres, it seems difficult to escape from the conclusion that substance has passed from the latter to the former. And still more convincing evidence may be adduced in the following manner: The two sartorii from the opposite legs of the same frog are dissected out with the utmost possible care and placed immediately in a solution containing 3000 parts water, 1 part methylene-blue, and 21 parts sodium chloride. They are kept in this solution at from 0° to 5° C. for from twenty-four to forty-eight hours, when they will be found to be more or less stained by the methylene-blue. One of these muscles is frozen and cut into transverse sections, which are immediately examined. The methylene-blue will be found to be almost exclusively contained in the peripheral rings of those fibres which have taken the stain. The other muscle is placed in 0.7 per cent salt solution at a temperature of 50° C. for two or three minutes. During this period it may be seen to shorten to about half its previous length and to become correspondingly thicker, in other words, to go into a true heat rigor. If it be now frozen and cut into transverse sections, the methylene-blue will be found exclusively contained in the central areas of its fibres. This experiment I have of course frequently repeated, and, whatever conclusions are to be drawn from it, it seems to leave no doubt of the fact that during the oncoming of heat rigor some substance which stains with methylene-blue passes from the periphery to the centre of the muscle fibres. Before the advent of heat rigor the methylene-blue is found in the peripheral rings, afterward in the central areas, and there seems no escape from the conclusion that while heat rigor is coming on, the blue-staining substance passes from one place to the other. I have not yet been able to obtain muscles stained with methylene-blue sufficiently irritable to contract markedly when stimulated with a tetanizing current; but I think that a consideration of the experiments so far described will leave no doubt that the movement of fluid from the periphery to the centre of the fibres is one of the phenomena of contraction.

The hypothesis gains still further support from a consideration of the more atypical forms of muscle fibres seen in cross sections prepared by the freezing process, all of which it readily explains.

A, *B*, *C*, *D*, and *E*, Fig. 4, are forms quite frequently seen in sections cut from frozen muscle. In *A* and *B* the blue-staining substance from a part of the peripheral ring has passed into the central area,

while another part of the ring has not been affected. In *A* the blue-staining substance is uniformly distributed throughout the central area, while in *B* it has only penetrated a part of the area, the remainder being left unstained. In *C* the blue-staining substance has passed in from all parts of the ring, but has not penetrated the whole of the central area; at *a* is seen an unstained area. These incomplete forms of contraction are frequently seen in sections cut from muscles caused to contract by a tetanizing current. *D* and *E* are most frequently seen in sections from muscles thrown into the extreme and abnormal form of contraction known as heat rigor. In these it seems as if not only the blue-staining substance had been taken into the central area, but after it the non-staining substance of the peripheral ring as well. In *D* this has been accomplished partially; in *E*, completely. The cross section shown at *B*, Fig. 4, seems to me particularly remarkable. The blue-staining substance appears like a cloud, partially filling the central area and shading off gradually into the unstained part of that area. The appearance is difficult to reproduce, but it gives an irresistible impression that the blue-staining substance from one side of the peripheral ring has partially penetrated the central area.

The facts to be learned from a study of cross sections of frog's muscle prepared by the freezing process are by no means the only available support for the view that the contraction of the fibres of this form of muscle is a process essentially similar to that described by McDougall for the fibrils of the wing muscles of insects. A large part of McDougall's argument rests on the evidence for the presence of inextensible transverse membranes in the fibrils of the insects' muscles. He believes that the contraction of the fibrils depends on the presence of these inextensible membranes in the same way that the shortening of the model shown by Figs. 1 and 2 depends on the presence of the inextensible wire rings *E*, *E*, *E*. The cross markings in frog's muscle fibres and in other forms of vertebrate muscle are much too close together to play this part in contraction. But the following experiment indicates that there exists in frog's muscle fibres a structure to which little attention has hitherto been paid, but which might very well have the function attributed by McDougall to the transverse membranes of the insects' wing fibrils. The living muscle of a frog is placed in a 2.5 per cent solution of glycerine in water. It is allowed to remain in this fluid at a temperature of from 0° to 5° C. for twenty-four hours; then transferred to 5 per cent gly-

cerine, in which it remains for another twenty-four hours at the same temperature; then transferred to 10 per cent glycerine; and so on up to 25 per cent or 30 per cent glycerine. If the fibres of this muscle be now teased out and examined in 30 per cent glycerine, great numbers of them will present the appearance of Fig. 5.

Grützner[1] describes this peculiar appearance in the fibres of the voluntary muscle of the rabbit, and gives excellent illustrations of it. He states that the substance in the light bands, *b*, *b*, *b*, Fig. 5, is more highly refractive and more strongly anisotropic than that in the rest of the muscle fibre; that it extends through the whole thickness of the fibre, as is shown by the fact that in cross sections of such muscles some of the fibres are altogether clear like the light bands, and others altogether dark; that the appearance is seen in the fibres of fresh normal muscles, which have been hardened with Müller's fluid, or treated with solutions of potassium chloride, or subjected to the influence of various other reagents; but that the appearance is seen most frequently in fibres from muscles treated with warm water or physiological salt solution. He is of the opinion that the light bands correspond to areas in which the muscle fibres have gone into heat or death rigor. I have repeated most of his experiments with frog's muscle, and have obtained the same results except in one case. I have not been able to produce the appearance in frog's muscle fibres by treating them with warm water or salt solution.

Longitudinal sections of muscle fibres presenting this peculiar appearance are extremely interesting. Such a section is shown by Fig. 6. I have of course made serial sections and followed the same fibre through several sections, so that I can state positively that the light band extends through the whole thickness of the fibre. Fig. 6 represents a section of thickness not more than one-seventh the diameter of the fibre, and cut through that portion of the fibre at which the diameter is greatest.

In sections from muscles prepared by the glycerine method described above, and then washed out thoroughly with distilled water, stained with carmine, dehydrated, and embedded in paraffin, the appearance is practically always that of Fig. 6. That is, each of the light bands is represented by a narrow light line, not more than 10 μ in width, and bounded on either side by a thicker deeply red line. The muscle substance is often more deeply stained in the

[1] GRÜTZNER, P.: Ergebnisse der Physiologie, Dritter Jahrgang, II Abtheilung, Biophysik und Psychophysik, 1904, p. 26.

neighborhood of these markings than in the areas midway between them.

I do not wish to dispute the suggestion that this remarkable appearance is largely due to post-mortem changes. The light bands do not appear in fibres teased out from a fresh living muscle, and therefore the fibres in which they do occur must have undergone some change. But it is impossible to suppose that the appearance teaches absolutely nothing in regard to the structure of the muscle fibre. The whole science of histology rests on evidence obtained from dead tissues subjected to treatment with various reagents; and if everything had to be given up except what can be learned from living organisms, there would be little left on which to build.

It must be admitted that the ratio between the diameter of the muscle fibre and the distance between two neighboring light bands is not constant. The extremes of the variation of this ratio are shown in *A* and *B*, Fig. 5. But the arrangement of the light bands is very far from irregular. Long series of them are seen at almost exactly equal distances from each other; and a very significant fact is that the bands are usually closest together in the thickest fibres. Again, the sharpness in the appearance of the individual markings, as seen in longitudinal sections (Fig. 6), indicates that they cannot be the effect of some chance decomposition. Grützner[1] indicates his opinion that the distance between the markings depends largely on the degree to which the muscle has been allowed to contract in the process of preparation; and it seems a far from improbable supposition that in completely extended muscle fibres the light bands would be at absolutely equal distances, and that the apparent variation is due merely to different degrees of contraction and to different degrees of swelling or shrinkage of the tissues under the influence of the reagents used in preparing them.

In regard to Grützner's suggestion that the appearance is the expression of oncoming rigor mortis or heat rigor, I can only urge the following objections: I have never been able to produce the appearance in frog's muscle by throwing it into heat rigor. This is very possibly due to some difference in technique, but it may at least be stated positively that frog's muscle fibres in heat rigor often do not show the light bands. If, as Grützner seems to indicate, the light bands represent areas in which rigor mortis has appeared, muscle fibres in complete rigor mortis should have the appearance of the light bands

[1] *Loc. cit.*

through their whole length; but as a matter of fact they have much more nearly the appearance of the intermediate darker areas. If the light bands are areas in rigor mortis, that is, in contraction, the muscle fibre should be thicker at their levels than elsewhere; but I have seen fibres showing long series of light bands and not bulged out at their levels at all. Indeed, the fibre is often seen to bulge more or less in the areas *between* the light bands (see Fig. 6). If Grützner's suggestion is correct, muscles showing the light bands should be more or less contracted; but I have found great numbers of fibres showing the light bands in muscles prepared by the glycerine method, and having by careful measurement a length equal to or a little greater than their length when living and fully extended. Finally, the fact that this appearance is seen in muscles treated in so many different ways seems to indicate that it has something to do with the structure of the muscle fibre.

Enough has been said to show the probability that the structure of the muscle fibre is differentiated at intervals corresponding to the points at which the light bands appear. Some such structural differentiation has been already indicated by the appearance I have described in muscle fibres in water rigor and in muscle fibres contracting under the influence of an electric current;[1] and still further light is thrown on the physical nature of the light bands by the following facts: If fibres be teased out from a muscle treated with glycerine by the method described above and carried up to a strength of 30 per cent, and if these fibres be then immersed in concentrated glycerine, the outlines of each fibre will be markedly drawn in between the light bands, giving the appearance of Fig. 7.

The reason for this drawing in of the outlines of the fibre is very evident. The osmotic pressure of the concentrated glycerine, in which the fibre is immersed, is much higher than that of the 30 per cent glycerine, with which it is penetrated. There is consequently a strong tendency for fluid to pass out of the fibre, and for its diameter to become less at all points. As the diameter remains the same at the levels of the light bands, while decreasing at all other points, there is every reason to believe that they are stiffer than other parts of the fibre. A further hint as to their physical peculiarities is gained from the fact that the muscle fibre is often found broken across at their levels, and there can hardly be a doubt that they have some connection with the phenomenon of fragmentation,

[1] *Loc. cit.*

in which pathologists have lately been so much interested. It seems as if at these points the muscle fibre lost in longitudinal tensile strength what it gains in lateral inextensibility.

The reason for the foregoing discussion of the appearance of the remarkable distinct cross markings in the voluntary muscle fibres of the frog, will, I think, be evident to any one who understands the imbibition hypothesis. I have been trying to show that the contraction of frog's muscle fibres is a process essentially similar to that which McDougall describes for the fibrils of insects' wing muscles, and which may be roughly illustrated in the shortening of the model shown by Figs. 1 and 2. In my previous paper[1] it was shown that contracting fibres could often be seen to assume the form of the contracted model, and the well-known facts of water rigor were adduced to show that muscle fibres could be made to contract by artificially increasing the volume of their contents. A study of cross sections of contracted and uncontracted muscle fibres prepared by the freezing process seems to show directly that during contraction fluid passes into the muscle fibres, that is, into the central areas. The peripheral ring is of course only a reservoir of fluid ready to pass into the fibre proper, and, as shown by *E*, Fig. 4, disappears entirely when the fibre is completely contracted. It was still necessary to show the existence in frog's muscle of some structure which could be compared to the wire rings of the model, or to the inextensible membranes described by McDougall in the insects' wing fibrils. This gap in the argument is supplied by the appearance of the light bands, which thus constitute an important link in the chain of circumstantial evidence supporting the imbibition hypothesis.

A full discussion of the change of form which frog's muscle fibres undergo during contraction must be left for a later article. The study of muscle fibres hardened by various reagents and of longitudinal sections cut from muscles frozen in contraction has led me to the belief that the usual form of contracted fibres is more like that described by McDougall for the wing fibrils of insects than like that of the contracted model shown by Fig. 2. The difficulties of the subject may be inferred from a consideration of what has been said regarding cross sections of muscle prepared by the freezing process. It is of course the central part of the muscle fibre which corresponds to the wing fibrils of insects and to the rubber tube of the model. The change of form which this part of the muscle fibre

[1] *Loc. cit.*

undergoes during contraction is clouded by the surrounding peripheral ring of deeply staining substance, the form of which is more or less fortuitous. It is one of the merits of the imbibition hypothesis, as stated above, that it explains many of the extraordinary and apparently utterly irregular appearances of muscle fibres prepared by various methods; but it is quite impossible to enter on a discussion of this subject in the present article. Fig. 8 represents a fibre from a muscle hardened by osmic acid while under the influence of a tetanizing current. Many fibres from muscles treated in this way show a striking resemblance to various stages of contraction described by McDougall in the fibrils of insects' muscles.

Although it is impossible to consider properly the literature of the subject of muscle in this article, certain observations bear so directly on some of the statements I have made, that it will be worth while to mention them in the briefest possible compass. Schaffer[1] describes and illustrates ringed muscle fibres strikingly like those of Fig. 3, which he observed in cross sections of human muscle hardened in Flemming's solution. The muscle had been cut fresh from the gastrocnemius of an executed criminal, and placed immediately in the hardening reagent. Grützner[2] describes the appearance of light bands like those shown by Fig. 5, not only in the voluntary muscle of the rabbit, but also in the smooth muscle of the small intestine of the cat.

The beaded appearance of contracted muscle is described by many observers in many different forms of muscle. Dwight[3] has studied the living muscle fibres in the leg of one of the water beetles (Gyrinus), and states that during contraction "the outlines of these fibres always become scalloped." He gives an illustration of a contracted muscle fibre of which the appearance is not unlike that of Fig. 8. The fibre in Dwight's illustration shows a much more regular beading than that of Fig. 8. It is of course not to be expected that muscle fibres subjected to the usual histological processes should show more than a distant approach to the form of muscle fibres living and actively contracting. Biedermann[4] gives an illustration of the muscle fibre of a beetle (Aphodius rufipes) treated with strong

[1] SCHAFFER, J.: Sitzungsbericht der kaiserlichen Akademie der Wissenschaften zu Wien, 1893, cii, p. 7.

[2] *Loc. cit.*

[3] DWIGHT, T.: Proceedings of Boston society of natural history for 1873.

[4] BIEDERMANN, W.: Electrophysiologie, Jena, 1895, pp. 36 *et seq.*

alcohol and acid. This fibre shows an appearance not unlike that of Fig. 8, and Biedermann states that Rollett has often observed this form in fresh, living fibres, contracting beneath the microscope. Biedermann also states on page 19 that the beaded appearance has been observed in preserved specimens of the involuntary muscle fibres of the poison gland of the salamander and of the intestine of the cat.

I must conclude with a statement of the scope and purpose of the foregoing article. An adequate account of the reasons for accepting the imbibition hypothesis of muscular contraction would involve a review of the whole subject of the histology and physiology of muscle, which is, of course, utterly impossible in an article like this. But McDougall's articles have hitherto attracted so little attention that it has been necessary for me to give at least a simple statement of his hypothesis in order to make clear the meaning of my own observations. These latter I have stated in the shortest possible compass, almost without touching on their relation to the vast literature which has grown up in regard to the subject of muscle. They constitute but a small part of the whole body of evidence in favor of the imbibition hypothesis. This evidence is constantly accumulating and will, in the near future, it may be hoped, be presented to the world in a proper form.

EXPLANATION OF FIGURES.

FIGURE 3. — Two forms of fibres seen in cross sections of muscle prepared by the freezing process. *a*, deeply stained peripheral ring; *b*, lightly stained central area; *a'*, lightly stained peripheral ring; *b'*, deeply stained central area. The tissue is that of a summer frog.

FIGURE 4. — Atypical forms of fibres seen in cross sections of frog's muscle prepared by the freezing process. *a*, portion of central area not yet penetrated by the blue staining substance. The tissue is that of a summer frog.

FIGURE 5. — Muscle fibres prepared by treatment with gradually increasing strengths of glycerine. In *A* the light bands are shown at their greatest distances; in *B*, at their least distances. At *a* the ordinary cross striation is visible. *bbb*, light bands.

FIGURE 6. — Longitudinal section 15μ thick of muscle fibre about 140μ in diameter; stained with carmine. The tissue is that of a winter frog, and was prepared by treatment with gradually increasing strengths of glycerine.

FIGURE 7. — Muscle fibre prepared by treatment with gradually ascending strengths of glycerine, and finally treated with concentrated glycerine.

FIGURE 8. — Portion of fibre from muscle hardened by osmic acid while under the influence of a tetanizing current.

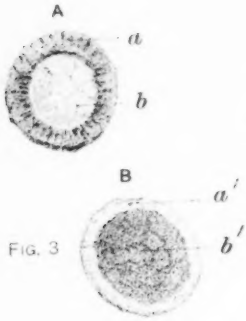


FIG. 3

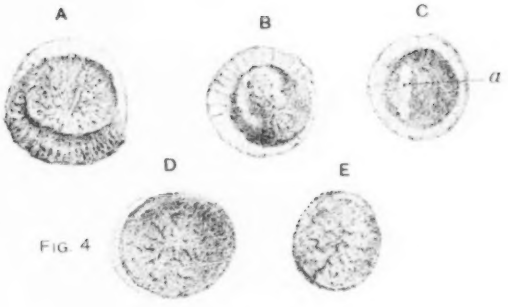


FIG. 4

0.2 M.M.

0.2 M.M.

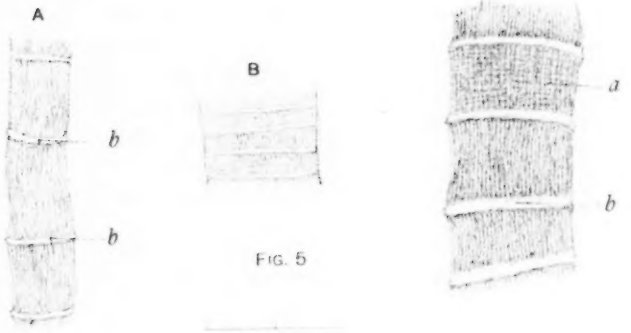


FIG. 5

0.2 M.M.

FIG. 6.

0.2 M.M.

FIG. 7

0.2 M.M.

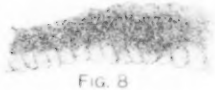


FIG. 8

0.2 M.M.

HELIOTYPE CO., BOSTON.

THE SOLUBILITY OF GLOBULIN IN SALT SOLUTION.

By THOMAS B. OSBORNE and ISAAC F. HARRIS.

[*From the Laboratory of the Connecticut Agricultural Experiment Station.*]

MANY protein substances which do not dissolve in pure water pass into solution on the addition of a neutral mineral salt. Proteins which are thus soluble in dilute saline solutions are known as globulins, and are found widely distributed in animal and vegetable tissues, being especially abundant in the seeds of nearly all the plants thus far examined. Although in some respects seed globulins differ from animal globulins, notably in not being precipitated, in most cases, by saturating their solutions with magnesium sulphate, nevertheless, they show in a very marked degree that most essential character of a globulin, solubility in saline solutions and insolubility in pure water. Many of these plant globulins are but slightly soluble at the room temperature in relatively strong saline solutions, *e. g.*, 2 to 3 per cent of sodium chloride, whereas they dissolve in very large proportion in those containing 5 to 10 per cent. The solubility of the globulin increases with the temperature, the increase above 30° being especially rapid. Concentrated solutions prepared at these higher temperatures, when cooled deposit the globulin either in crystals or in well-formed spherules or spheroids.

It is thus evident that we have in seeds protein substances which, in a typical manner, show the property of being dissolved by solutions of neutral mineral salts. The proportion of salt required to effect solution varies with the nature of the protein as well as with that of the salt. Some salts which, at a certain concentration, dissolve much of the globulin, at higher concentrations dissolve less, and at still higher concentrations dissolve none at all, so that a solution of the globulin prepared with a dilute solution of such a salt, is completely precipitated by adding crystals of the salt, until the concentration is raised to a certain degree. Ammonium sulphate precipitates all proteins, except peptones, when introduced into their solutions to complete saturation, and precipitates many when introduced up to a certain definite degree of concentration, which has

been considered to be characteristic for each. In consequence of this property, the separation of some of the different proteins has been effected, and the limits of concentration between which this precipitation begins and is completed have been established for them. The behavior of most of the globulins toward saturated solutions of magnesium sulphate, sodium sulphate, and sodium chloride has also been determined and recorded. Nothing, however, is known of the nature of the process by which the salt effects solution of the protein, and the subject, though it has frequently attracted attention, has been but little studied.

Pauli[1] states that non-electrolytes, such as sugar and urea, have no solvent action on globulins, and supposes that electrolytes effect solution by the union of the two ions of the salt with different parts of the protein molecule, thereby forming a soluble compound. Cohnheim[2] remarks on this view that the fact that the globulin is precipitated by dilution from its saline solutions, whereby no diminution of its ions occurs, is not thus accounted for. This criticism of Cohnheim's, however, is not applicable, for although it is true that the actual number of ions is increased by dilution, their cencentration is greatly diminished, a fact which may have much to do with the precipitation.

Furthermore, the power to dissolve globulin at neutral reaction is not limited to electrolytes, as Pauli supposes, for Ramsden[3] has recently shown that concentrated solutions of urea have a powerful solvent action on many kinds of proteins, including globulins, and alcohol has long been known to be an energetic solvent for many of the proteins of the cereals. We have been able to confirm Ramsden's observation with edestin, which at once yields a perfectly clear and bright solution when treated with a concentrated solution of urea.

Starke[4] has stated that alkaline reaction given by a small quantity of sodium hydroxide was stronger in a sodium chloride solution than in an equal volume of pure water, and to this he attributed the solvent action of salts upon the globulin which he supposes is brought into solution as an alkaline compound. He, however, considered only the globulins of animal origin, which, in nature, occur in solutions alkaline towards litmus.

[1] PAULI: Archiv für die gesammte Physiologie, 1899, lxxviii, p. 315.
[2] COHNHEIM: Die Eiweisskörper, Braunschweig, 1900, 150.
[3] RAMSDEN: Journal of physiology, 1902, xxviii, p. xxiii.
[4] STARKE: Zeitschrift für Biologie, 1900, xl, p. 419, and 1901, xlii, p. 187.

In the seeds of plants are found large quantities of protein substance, which, as we have already stated, show in a marked degree the properties characteristic of globulin. These can be obtained by treating the ground seeds with solutions of pure, neutral sodium chloride, in which they dissolve with a distinct acid reaction towards litmus. By dialysis or dilution the plant globulins are precipitated, and, after washing and re-dissolving in neutral salt solution, yield a solution which reacts decidedly acid with litmus, and more so with phenolphthalein.

Such globulin solutions certainly do *not* contain an alkali-protein compound, but, as the writer has shown, an acid compound of the globulin, *i. e.*, a protein salt.[1]

When dissolved in neutral sodium chloride solution, and precipitated by carbonic acid, edestine separates as chloride, and a corresponding amount of sodium carbonate remains in solution.[2] In this case the globulin does not combine with the positive ion, but with the negative, as would be expected from the now recognized basic properties of the protein molecule.

Furthermore, edestin, dissolved in water with a minimal quantity of alkali, forms a solution which is abundantly precipitated by minute quantities of sodium chloride, in just the same way as solutions similarly made with acid are precipitated, the precipitate in each case being dissolved by a larger proportion of salt.

Starke lays much stress on the supposed fact that alkaline solutions of globulin are not thus precipitated, but this is certainly not true in the case of edestin. He also considers that the product which is obtained by precipitating globulin from saline solutions by carbonic acid, dilution or by dialysis, and which is then no longer soluble in saline solutions, is *alkali-free* protein, which, before precipitation was held in solution as an alkali compound. His ground for this opinion is that this product becomes soluble in sodium chloride solution after treating with dilute sodium carbonate solution. It seems to us more probable that this substance is a product similar to the edestan which one of us has shown to be formed from edestin by hydrolysis,[3] and which is much less soluble in the various solvents than the original globulin.

[1] Osborne: Zeitschrift für physiologische Chemie, 1901, xxx, p. 240.

[2] Osborne: This journal, v, p. 180.

[3] Osborne: Report of the Connecticut Agricultural Experiment Station for 1900, p. 388; Zeitschrift für physiologische Chemie, xxxiii, p. 225.

However true Starke's observations may be for the special cases which he examined, his conclusions certainly have no such wide application as he supposed.

As no one, so far as we know, has ever attempted to study the solubility of a globulin quantitatively, we give in the following pages the results of some experiments in this direction, made with the globulin edestin, which seem to afford a starting point for a further study of this question of such physiological interest and importance.

Edestin from the hemp-seed was chosen for these experiments because it is readily prepared in a pure crystalline state, is wholly insoluble in water, and forms definite crystalline salts with mineral acids which the writer has extensively studied, and of which the properties and composition are already ascertained.

The plan of the experiments was to suspend in a glass stoppered bottle 2 gm. of the air dry preparation, in water enough to make just 20 c.c. with the quantity of molar salt solution which was afterwards added. After agitating the mixture for some time at 20°, the undissolved globulin was allowed to settle, and then 10 c.c. of the clear solution drawn out with a pipette, its nitrogen content determined, and from this the amount of dissolved edestin was calculated. A series of determinations was made, using successively larger quantities of the molar salt solution, and correspondingly less water, so that the number of molecules of the salt in each 20 c.c. portion was successively greater. In every case the amount of edestin was in decided excess of that dissolved, so that as nearly complete saturation of the solution as was possible, under the conditions of the experiment, was attained.

Although the results obtained in many instances show wide numerical differences, when judged by the usual standards of accuracy of chemical or physical determinations, they nevertheless are sufficiently accordant to afford a valuable comparison between the different classes of salts, and for the first time give us an opportunity of stating approximately their relative solvent power.

The minor differences indicated by the solubility are, without doubt, due to experimental errors, since it was not possible for us to so control the conditions as to wholly eliminate the influence of several disturbing factors.

One of these was the difficulty of completely saturating the solution with edestin, but we think that, in general, very nearly complete saturation was attained, because the edestin preparations were extremely

fine, dusty powders, composed entirely of microscopic crystals, which could be uniformly suspended in water, so that, after adding the salt solution and agitating for a short time, almost, if not quite, complete saturation soon resulted. That solution takes place almost immediately was shown by the fact that when enough of the salt solution was used to dissolve all but a very small part of the edestin, all except this latter dissolved at once, and the amount of undissolved edestin did not visibly diminish on longer shaking. In fact, solution takes place so rapidly as to indicate an ionic reaction. The uniform, very fine division of the substance in the minute microscopic crystals, each separately suspended in water, would, however, undoubtedly lead to very rapid solution.

Another difficulty was presented by the fact that the solutions could not be filtered, as, when saturated with the globulin, they were very sensitive, being rendered turbid by passing through a filter of even the purest paper. It was necessary, therefore, to draw off one-half of the solution with a pipette, after the undissolved edestin had settled, and it was not always possible to do this without disturbing the deposit. However, it rarely happened that more than insignificant quantities of the insoluble matter were drawn off with the solution.

Another source of error lay in the necessity of determining the amount of dissolved edestin from the nitrogen content of one-half of the solution, whereby any error in the nitrogen determination when expressed in terms of edestin was multiplied by ten.

Variations in the temperature of the solutions caused differences in the amount of edestin dissolved, but no serious error in our experiments resulted from this cause, as we found that variations of even two or three degrees did not seriously affect the result, and the temperature was easily kept constant well within these limits.

That these difficulties did not wholly destroy the value of the experiments is shown by the approximate regularity of the curves representing the solubility of the edestin in the several salt solutions, as well as the fair agreement between those made with the same or chemically similar salts.

This is illustrated by the curves given in Fig. 1, in which the results of determinations at 20° and 25° may be compared, and also the agreement between duplicate determinations made with sodium chloride solutions with the same as well as with different preparations of

edestin, the number given with each line indicating the preparation used. From this figure it is evident that an increase of 5° in the temperature causes a distinct increase in solubility, but the amount of this increase is such that the error caused by unavoidable differ-

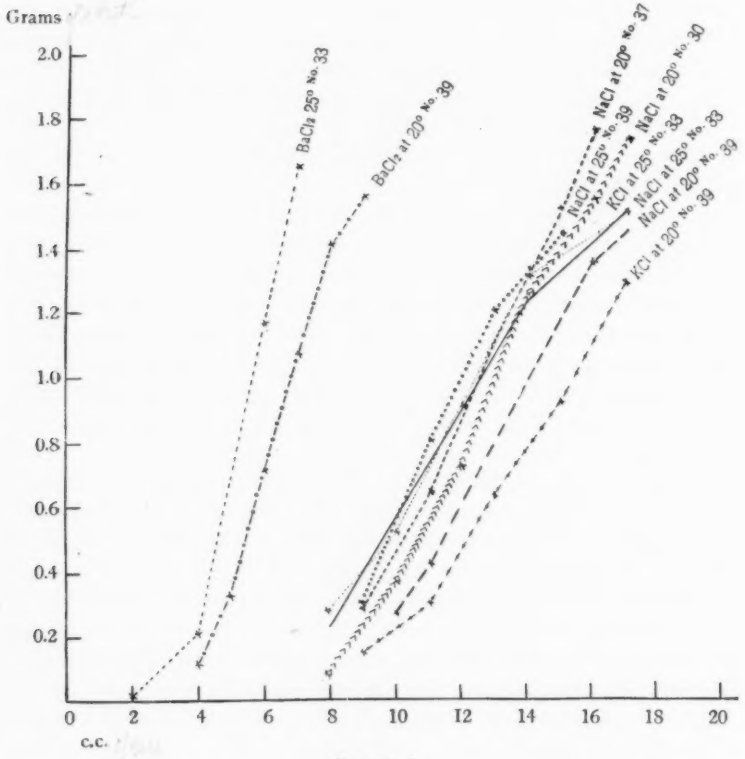


FIGURE 1.

ences in the temperature is not sufficient to materially affect the results. There appears to be too little difference in the solubility of different preparations of edestin to interfere with a comparison of the solvent effect of the different salts.

The writer has shown[1] that edestin with very small quantities of

[1] OSBORNE: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 240; also Journal of the American Chemical Society, 1902, xxiv, p. 39.

acid forms salts which, though crystallizing in the same form as the free edestin, differ from each other in their solubilities. We have,

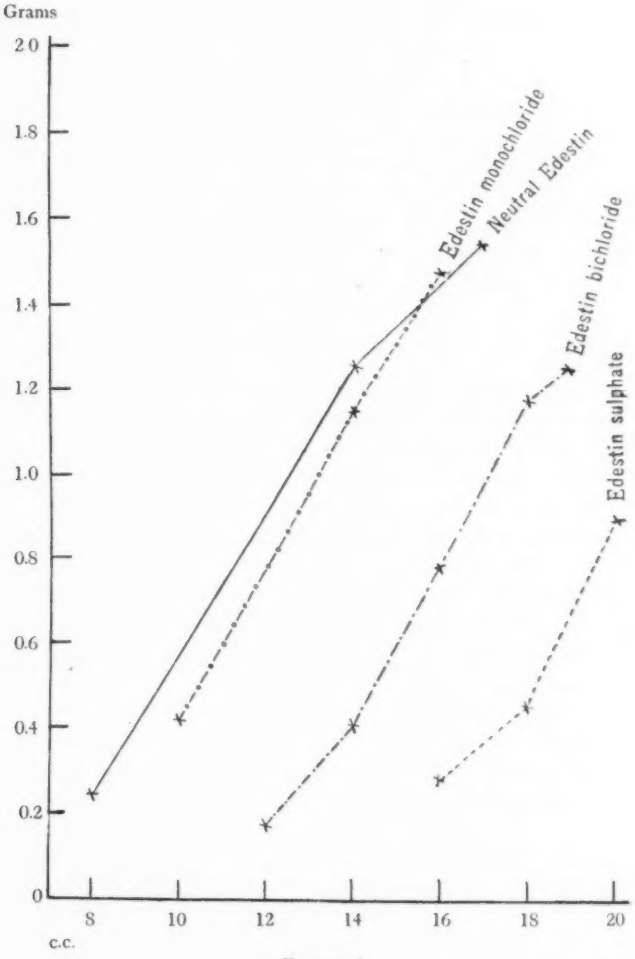


FIGURE 2.

therefore, determined the solubility of such salts of edestin in normal sodium chloride solution at 25° with the results given in Fig. 2, from which it is evident that while the amount of combined acid present in

the monochloride of edestin has but little effect on the solubility of this globulin in sodium chloride solution, that combined in the bichloride and the sulphate decreases the solubility to a very marked extent.

In the experiments next to be described we have, therefore, always used very carefully prepared edestin which had been repeatedly recrystallized from warm solutions of pure sodium chloride, and finally from one made perfectly neutral to phenolphthalein by the addition of a calculated quantity of decinormal sodium hydroxide solution, diluted with a very large quantity of water; after thus neutralizing, the edestin was carefully protected from the carbonic acid of the air until it had been thoroughly dehydrated with absolute alcohol. The preparations were made exactly according to the description given by the writer in the paper just referred to.[1]

Solubility of Edestin in Solutions of Chlorides.

The solutions used contained as many grams of the salt in 1 litre as there are units in its molecular weight, that is, the solutions of the chlorides of the monovalent bases were normal, those of the divalent bases twice normal, the object of the experiment being to compare the solvent power of the salts molecule for molecule.

Fig. 3 shows the results of experiments with such solutions made at 20°. The chlorides of the monovalent bases sodium, potassium, and cæsium[2] have very nearly the same solvent power as one another, which is only one-half that of the divalent bases barium, strontium, calcium, and magnesium, that is, the solubility is independent of the nature of the base, and proportioned to the chlorine atoms. To this generalization, however, there is one striking exception, namely, lithium chloride, which has a much lower solvent power than that of the other monovalent chlorides. No explanation of the anomalous behavior of this chloride has as yet been discovered.

Solubility of Edestin in Solutions of Sulphates.

Fig. 4 gives the results of experiments with solutions of sulphates from which it is evident that for most of these salts the solubility is nearly the same as in solutions of the corresponding chlorides.

[1] Osborne: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 265; and Journal of the American Chemical Society, 1902, xxiv, p. 58.

[2] We are indebted to Prof. H. L. Wells of Yale University for a large quantity of extremely pure cæsium chloride, for which we wish here to express our thanks.

It is well known that sodium sulphate precipitates most protein substances when introduced into their solutions in sufficient quantity. This precipitating effect is well seen in the curve given for this salt, which

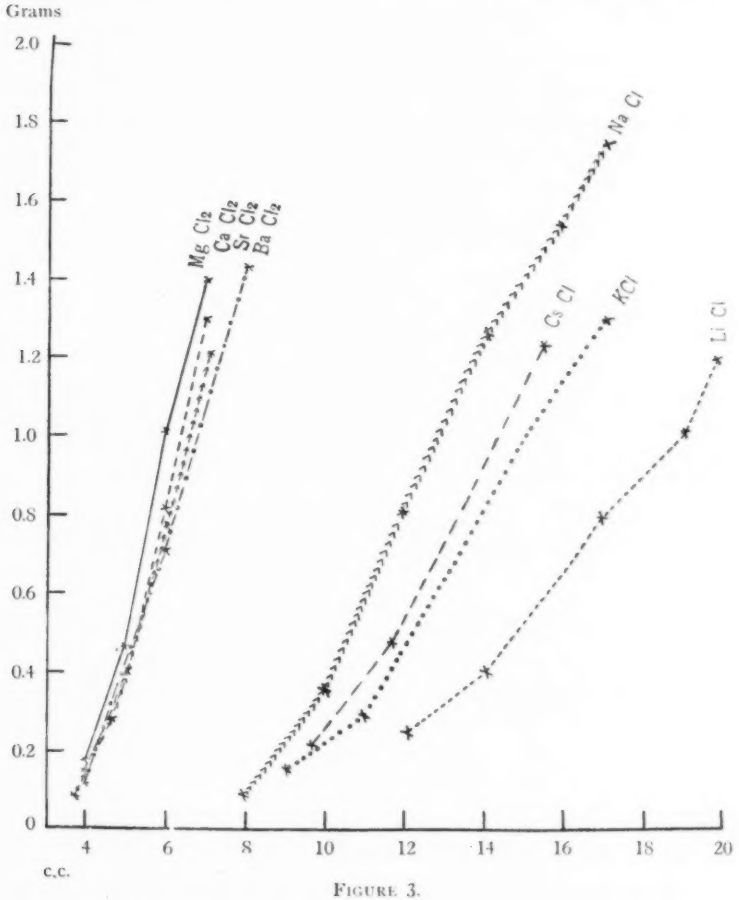


FIGURE 3.

shows that while a half molar solution dissolves the edestin as freely as any other salt, under similar conditions, a full molar solution dissolves scarcely any. With potassium sulphate the same effect is noticed, but owing to the limited solubility of this sulphate, actual precipitation does not take place. In experimenting with this salt we used a saturated

solution, and calculated the amount of salt present to the corresponding quantity of a molar solution. It is to be noted that the curve with potassium sulphate is extended beyond 14.5 c.c., which corresponds to a saturated solution of this salt. This extension was effected by

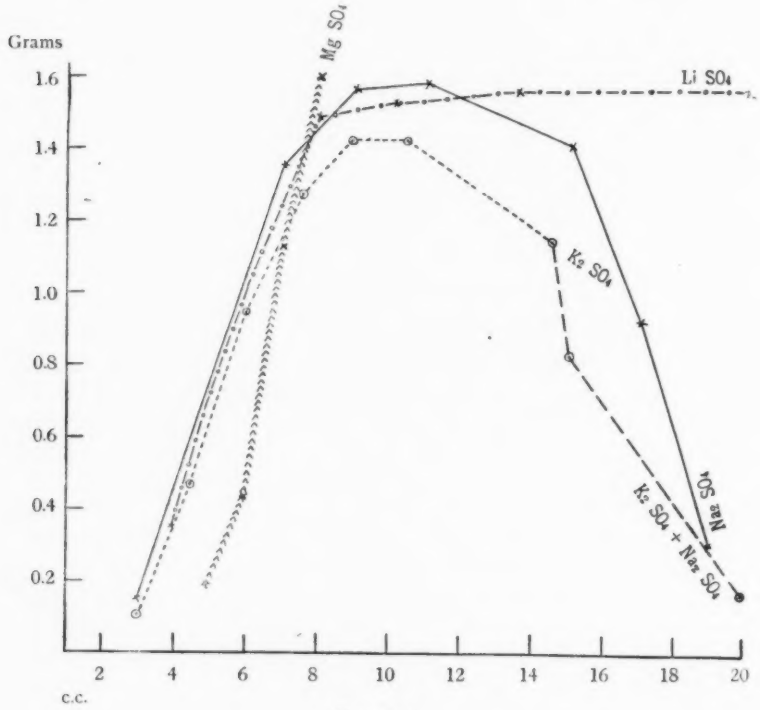


FIGURE 4.

using a solution containing an equal number of molecules of potassium and sodium sulphate. With this solution the curve follows very nearly the same course as followed by solutions of sodium sulphate of equal molecular concentration, so that it would seem to be true that potassium sulphate would have the same precipitating power as sodium sulphate were it sufficiently soluble in water. The curve for lithium sulphate follows those of potassium and sodium sulphate very closely indeed until the latter begin to fall. With lithium sulphate, however, the amount of dissolved edestin remained practically constant

with increasing concentration of the salt solution, and no evidence of diminishing solubility appeared.

Magnesium sulphate is commonly considered to precipitate globulins, when added to their solutions in sufficient amount, but this is not the case with edestin and many other vegetable globulins, which nevertheless are to be considered true globulins, if insolubility in water and ready solubility in solutions of neutral salts is to be taken as the essential characteristic of this class of substances. The lack of precipitating effect of magnesium sulphate is shown by the line given for this salt in Fig. 4, which follows the same course as that taken by solutions of the chlorides of divalent bases.

Solubility of Edestin in Solutions of Bromides and Iodides.

Fig. 5 gives the results of experiments with these salts, from which it appears that solutions of sodium and potassium iodide have each the same solvent effect, which is much greater than that of solutions of the bromides of the same molecular concentration, and slightly greater than that of the chlorides of divalent bases. Both sodium and potassium bromide show nearly the same solvent power, which is considerably higher than that of corresponding solutions of the chlorides. The bromides of barium and calcium have each practically the same solvent power, but this is only a little greater than that of the bromides of the monovalent bases, and distinctly less than that of the chlorides of the divalent bases or of the sulphates.

Lithium bromide, like lithium chloride, has less solvent power than the corresponding solutions of sodium and potassium bromide, but the same solvent power as solutions of sodium chloride of the same molecular concentration.

Solubility of Edestin in Solutions of a Strong Base with a Weak Acid, and of a Strong Acid with a Weak Base.

The solutions already described have contained salts which are perfectly neutral to phenolphthalein. Fig. 6 shows that the solvent power of molar solutions of potassium chromate, sodium sulphate, and sodium thiosulphate is in the order named, and that all three are much more energetic solvents for edestin than any of the salts yet described. These three salts are hydrolytically dissociated with an alkaline reaction, and approach, in solvent power, sodium

carbonate, which has long been recognized as a powerful solvent for protein substances. If, on the other hand, we compare the solvent

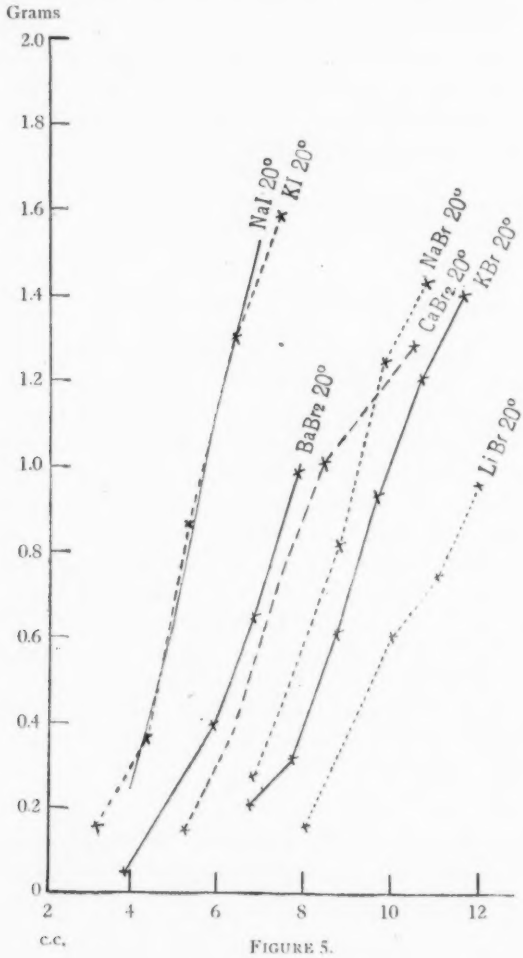


FIGURE 5.

power of manganese and ferrous sulphate, it is seen that these are less efficient than the sulphates of the strong bases, doubtless owing to a slight acidity of their solutions caused by hydrolytic dissociation. With manganese chloride solutions the results were complicated by

an alteration of the dissolved edestin which took place in the solutions containing the larger quantities of this salt. This alteration was accompanied by a change in the direction of the line, showing the sol-

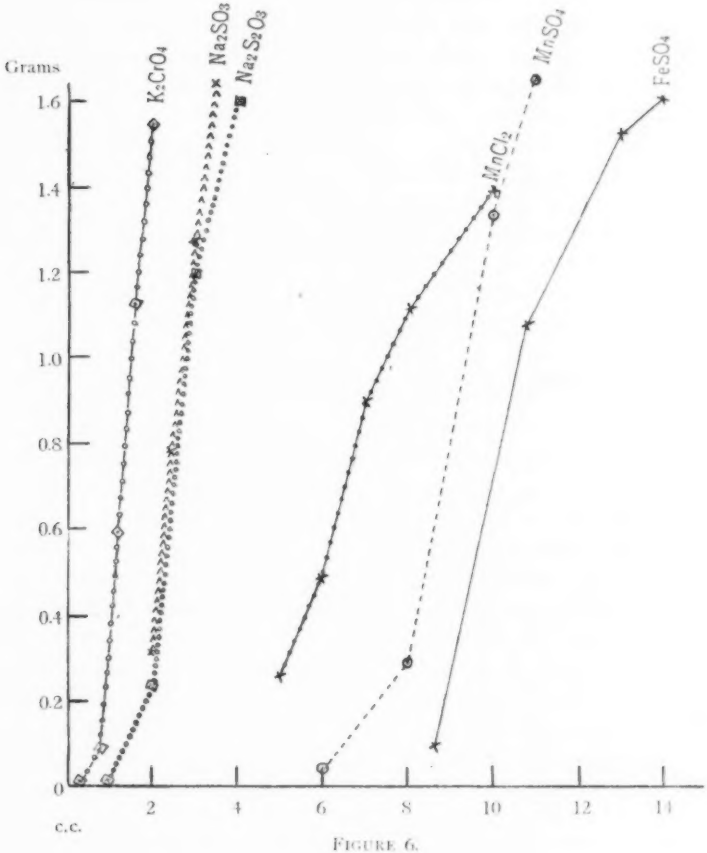


FIGURE 6.

ubility in solutions of this salt when more than 7 c.c. of the molar solution were used. With less than 7 c.c. the line agrees closely with that found for chlorides of divalent bases, but above falls rapidly away. In harmony with this, it was noticed that, on standing, the solution, with 8 c.c. and 10 c.c., gradually yielded a flocculent precipitate, which

accumulated in notable amount on top of the deposit of undissolved edestin. This precipitate was found to be insoluble in sodium chloride solutions, and is doubtless *edestan*, which the writer has shown [1] to be rapidly formed from edestin under the action of small quantities of acids. With 7 c.c. only an insignificant amount of this substance was formed, even after standing for three hours. None of this insoluble product was observed in the solutions containing less than 7 c.c. of the manganese chloride solution, nor was a similar change detected in solutions made with manganese sulphate.

That solutions of manganese and ferrous salts should thus dissolve edestin is surprising, for solutions of the chlorides and sulphates of the other heavy metals, so far as we have examined them, have no solvent effect whatever. The solutions of edestin produced by these manganese and ferrous salts appear to be of quite the same order as those yielded by the other salts described in this paper, since by dilution the edestin is precipitated from them, apparently unchanged.

Solubility of Edestin in Solutions of Acetates.

Edestin behaves towards acetates in a wholly anomalous manner. Solutions of sodium, potassium, or ammonium acetate, in every degree of concentration, dissolve none whatever, whereas the acetates of the alkaline earths have nearly the same solvent power as their chlorides, while magnesium acetate is distinctly less efficient than its chloride, or the acetates just named. The solvent powers of the acetates of barium, strontium, calcium, and magnesium are shown by Fig. 7 to be in the order named, that is, in the order of their molecular weights. Manganese acetate behaves like barium acetate, though its solvent power is much greater, as shown by Fig. 7. The experiments with this salt, however, are not satisfactory, owing to the difficulty of preparing a neutral solution of the acetate. The solution of the pure crystallized salt was distinctly acid to phenolphthalein, and could not be made neutral without precipitating the manganese. The solution used was made with pure crystallized salt, and neutralized to *litmus* with a little sodium hydroxide solution. At this point the solution became somewhat darker colored, probably from the formation of a basic salt.

The solution of edestin made with this solution of manganese acetate is precipitated by dilution with water.

[1] Osborne: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 225.

In contrast to their salts with strong mineral acids, which will be described later, the acetates of the metals, silver, lead, and copper, have very strong solvent properties, far exceeding any of the salts yet examined. The solvent power of each of these three acetates is the

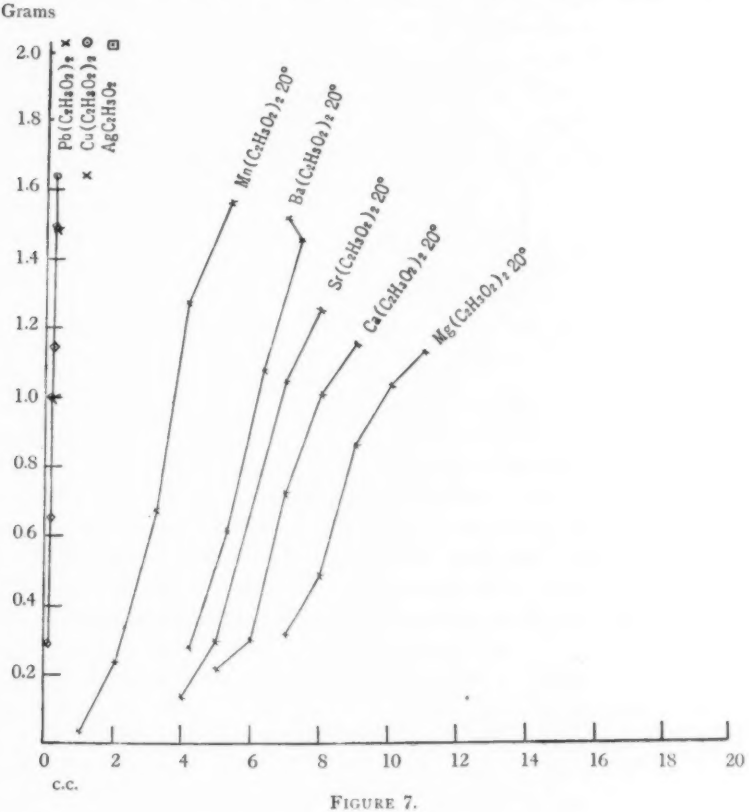


FIGURE 7.

same, and closely approaches that of free hydrochloric or acetic acid, as described in a previous paper.[1] Solutions of these acetates are such active solvents that the actual experiments were made with one-tenth molar solutions in order to obtain acurate results.

The metallic ion evidently combines with the edestin, as reactions for the free ions of these metals could not be obtained in the edestin

[1] OSBORNE: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 240.

solutions made with these salts. Thus the solution of edestin by copper acetate has a deep blue color, as strong as that given by the corresponding quantity of copper acetate solution with ammonia.

The solution by silver acetate gives a precipitate with a very little hydrochloric acid, soluble completely in a somewhat larger quantity, and under these conditions gives no silver chloride whatever.

The solution in lead acetate, when dialyzed in distilled water until free from any excess of lead acetate, yields a clear transparent jelly when further dialyzed into alcohol. The ash of this jelly contains much lead, showing the metal to have been in combination with the edestin.

Solutions of edestin made with these acetates behave like those made with pure acid. They are not precipitated by dilution with water, nor by a considerable quantity of alcohol, but with a very large excess of the latter yield voluminous transparent jellies.

The addition of a little sodium chloride or sodium acetate solution gives a large precipitate, just as in a solution of edestin made with a little hydrochloric acid. Conversely, solutions of edestin in sodium chloride are precipitated by a little copper acetate solution just as by a free acid.

The solution in lead acetate is alkaline to litmus, but distinctly acid to phenolphthalein, the acidity of the solution to this latter indicator being far greater than that of the acetate solution alone. Thus when 1 gm. of edestin was dissolved in 18.7 c.c. of water containing 1.3 c.c. of 1/10 molar lead acetate solution, it was necessary to add 1.3 c.c. of decinormal potassium hydroxide solution in order to neutralize the acid reaction to phenolphthalein, while 0.1 c.c. was sufficient to give a strong red reaction with the same amount of the acetate solution alone. The edestin solution behaved as if one-half of the $C_2H_3O_2$ ions were present as free acid. In harmony with this the solubility of the edestin was the same as in a solution containing this amount of free acetic acid.

In this connection it is interesting to note that the solvent power of silver acetate, with one-half as many $C_2H_3O_2$ ions, is the same as that of the copper and lead acetates. The solubility of the edestin in these metallic acetate solutions is of an entirely different order from that in the other salts already described, for a relatively large proportion of these other salts is required to dissolve corresponding quantities of edestin, and from these solutions the edestin is precipitated unchanged by diluting with water.

Solutions of other metallic acetates, such as those of zinc and mercury, do not dissolve edestin, but like most other salts of the heavy metals, act just like a mixture of a neutral salt and free acid. By these the edestin is converted rapidly into a curdy mass, no longer soluble in saline solutions.

Solubility of Edestin in Ammonium Salts.

As these salts contain nitrogen, the amount of dissolved edestin cannot be determined from the nitrogen content of the solution. We have, however, found that the solubility in ammonium chloride solution is practically the same as in sodium chloride, by comparing the undissolved residues which remain on treating the edestin with equimolecular proportions of the two salts; moreover, ammonium acetate, like sodium acetate, does not dissolve any edestin at 20°.

Solubility of Edestin in Solutions of Nitrates.

By treating 2 gm. of edestin, air-dried, with different quantities of the molar solutions of the nitrates, together with water enough to make a total volume of 20 c.c., we found the quantity required to dissolve all but an insignificant amount of the protein. That this was the least amount required, was shown by the larger residue of undissolved edestin that remained when 1 c.c. less of the nitrate solution was used. The amount of edestin thus assumed to be dissolved was about 1.7 gm. To accomplish this result we found that 9 c.c. of a molar solution of potassium or sodium nitrates were necessary, and 11 c.c. of strontium nitrate.

The limited solubility of barium nitrate is such that only a little edestin is dissolved by a saturated solution at 20°, and no quantitative experiments therefore were tried with this salt.

Solubility of Edestin in Solutions of Metallic Salts.

Besides those metallic salts already described, we have tested the following, none of which dissolve the edestin, but convert it into a curdy mass, apparently behaving in the same way as a mixture of sodium chloride and hydrochloric acid: Copper, cadmium, chromium, cobalt, ferric and lead nitrates, mercuric, copper, aluminum, zinc, and cadmium chlorides, and zinc and copper sulphates.

Ferric chloride behaves differently, as it dissolves edestin freely, the resulting solution not being precipitated by dilution, nor by much

alcohol, nor by an excess of ferric chloride. By a very little sodium chloride, the protein is precipitated from this solution. By a slight excess of hydrochloric acid, it is not, by a little more it is precipitated; by still more the precipitate is redissolved, and by a larger quantity is again thrown down. This solution in ferric chloride behaves much like a solution in free acid.

TABLE I.

	KSCN in 25 parts water.	AgSCN in 25 parts water.
15, 16	9.93	6.69
14	10.47	6.96
13	16.54	9.33
12	40.9	16.79
10, 11	43.9	17.68
9	45.5	14.98
8	51.9	12.05
7	56.7	10.89
6	64.7	10.66
5, 4, 3	68.9	9.66
2, 1	59.8	0

The following note respecting the similarity of the solution of edestin in solutions of neutral salts with that of a mineral salt in solutions of other mineral salts has been kindly furnished by H. W. Foote, assistant professor of physical chemistry in Yale University, to whom we are also indebted for several helpful suggestions which he has made during the progress of this work.

"The solubility of edestin in salt solutions is not unlike the solubility of certain insoluble inorganic salts in solutions of other salts. In the latter case the solubility is due to the formation of one or more addition products, or complex salts in solution. It seems not unreasonable to assume that addition products of the edestin and salt are also formed in solution, a suggestion which has already been made by Pauli.[1]

[1] PAULI: Archiv für die gesammte Physiologie, 1899, lxxviii, p. 315.

"The solubility of silver thiocyanate in solutions of potassium thiocyanate has previously been determined.[1]

"To show that the curves are not unlike some of those obtained for the solubility of edestin in salt solutions, the solubility results have been recalculated to give the parts of silver thiocyanate and of potassium thiocyanate in twenty-five parts of water. The results are in Table I. The numbers in the first column refer to the corresponding results in the article as originally published.

"These values are plotted in Fig. 8. Potassium thiocyanate is plotted as abscissa, corresponding to the salts used to dissolve edestin, and silver thiocyanate as ordinate, corresponding to the edestin. The

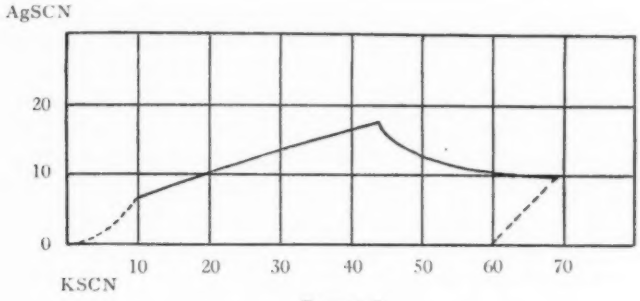


FIGURE 8.

form of the first part of the curve has not been directly determined, so it is indicated as a dotted line. That it is certainly concave as represented is shown by the fact that silver thiocyanate is quantitatively insoluble in dilute potassium thiocyanate solutions.

"The different parts of the curve show where different solids were present. Silver thiocyanate was present along the dotted line at the left, and potassium thiocyanate along that at the right. One double salt containing silver and potassium thiocyanate was present along each of the other two parts of the curve.

"In the same way, along any of the curves which do not show a sharp break in the direction it is probable that either edestin or a compound with the salt is present in the solid form. Where a distinct change in the direction of the curve is found, as with the sulphates of sodium, lithium, and potassium, it is probable that a

[1] H. W. FOOTE, American chemical journal, xxx, p. 330 (193).

different solid is present, perhaps a compound of edestin with the corresponding sulphate."

Conclusions.

1. It is possible to determine quantitatively the solvent power of salt solutions on the globulin edestin with sufficient accuracy to obtain valuable information respecting the relative solvent powers of these salts.

2. Two distinct forms of solution result where saline solutions are applied to edestin, one which requires the presence of a relatively considerable quantity of salt before notable quantities of the globulin dissolve, and from which the edestin is precipitated unchanged by dilution with much water, as well as by the addition of small quantities of strong acids; the other, which is caused by relatively very small quantities of the salt, is not precipitated by dilution with water or by dialysis, nor by small quantities of acids, and from these solutions the edestin has not been separated unchanged, as the positive ion enters into combination with it. Solutions of the first kind are produced by neutral salts of strong bases with strong acids, those of the second kind by solutions of salts of weak bases, and therefore closely resemble solutions in pure acids.

3. Saline solutions of edestin are strongly influenced by the presence of minute quantities of other substances, especially bases and acids; hence, the statements here made apply only to *pure* edestin in solutions of the pure salts.

4. The primary compound of edestin with acids, that is, the salts of edestin such as the chlorides and sulphates, are less soluble than the free edestin.

5. Equal quantities of normal solutions of most salts of strong bases with strong acids have equal solvent power; those tested and found to follow this law were sodium, potassium, cæsium, ammonium, barium, strontium, calcium, and magnesium chlorides, and sodium, potassium, lithium, and magnesium sulphates. Those found to be exceptions were the nitrates of sodium, potassium, calcium, and strontium; the chloride of lithium, the bromides of sodium, potassium, barium, calcium, and lithium, and the iodides of sodium and potassium.

6. The solubility of edestin in solutions of salts of strong bases with strong acids resembles the solubility of certain insoluble inorganic salts in solutions of other salts, in consequence of the formation of complex salts in solution. It seems reasonable, therefore, to assume

that soluble addition products of the globulin and salt are also formed, a suggestion which has already been made by Pauli.

7. Salts of strong bases with weak acids have a greater solvent power than similar salts containing strong acids. The solvent power of such salts was found to be in the order named: Sodium carbonate, potassium chromate, sodium sulphite, and sodium thiosulphate.

Salts of weak bases with strong acids have a lesser solvent power than similar salts with strong bases. The solvent power of such salts was in the following order: Manganese chloride, manganese sulphate, and ferrous sulphate.

8. The behavior of edestin towards acetate solutions is anomalous.

Although it is insoluble in solutions of potassium, sodium, or ammonium acetate, it is nearly as soluble in solutions of barium, strontium, calcium, and magnesium acetates as in solutions of the chlorides of these latter bases. The solubility in solutions of these four acetates is in the order of their molecular weights.

In solutions of silver, copper, and lead acetates it is nearly as soluble as in solutions of free acid of corresponding concentration. The solutions made with these acetates closely resemble those made with acids. The positive ion enters into combination with the edestin, and no longer remains as a free ion in the solution. Acetates of zinc and mercury have no solvent action.

9. Salts of the heavy metals combined with strong acids behave like a mixture of a neutral salt with free acid, as, for example, a mixture of sodium chloride and hydrochloric acid. Ferric chloride is an exception, behaving much like free hydrochloric acid.

PUBLICATIONS FROM THE NEW YORK STATE CANCER LABORATORY:
H. R. GAYLORD, G. H. A. CLOWES, G. N. CALKINS.

NO. 32. ON THE RELATIONSHIP BETWEEN THE RATE OF GROWTH, AGE, AND POTASSIUM AND CALCIUM CONTENT OF MOUSE TUMORS (ADENO-CARCINOMA, JENSEN).

BY G. H. A. CLOWES AND W. S. FRISBIE.

THIS communication deals with one phase of a general investigation being carried out at the present time on the Jensen tumor mice by the staff of this laboratory. The original tumor from which this series has been derived was discovered and successfully transplanted through several generations of mice by Professor Jensen of Copenhagen, through whose courtesy a couple of mice were placed at the disposal of the staff of this laboratory in February, 1904. The mice were brought to Buffalo by Dr. Gaylord, the tumors removed, admixed with normal salt solution in the proportion of 1 : 3, and injected subcutaneously into a couple of series of normal mice. Several tumors developed in one of these batches and afforded material for further transplantation and experiment.

Up to this time about 1600 mice have been employed in this laboratory in the experimental work directed towards the determination of the conditions influencing the development of these tumors. Excluding those which have been employed for experiments in which the use of filtration, high temperatures, chemicals, etc., previous to inoculation, might be expected to interfere with the normal development of the tumor, also those cases in which some type of infection or other accident led to the death of mice shortly after inoculation, it may be said that approximately 1100 mice have yielded about 450 tumors, of which number over 300 have developed and caused the death of the mice.

The results of certain preliminary immunity experiments affording evidence of the existence in spontaneously recovered mice of forces

antagonistic to the development of the tumors are to be found in the communications from this laboratory, Nos. 27, 28, and 31.[1]

Previous to December, 1904, the number of tumors at our disposal was too small to permit of their being employed for the determination of mineral constituents, and experiments made with a view to isolating and identifying certain organic constituents had to be abandoned for the time being, on account of the extremely small size of the tumors, which seldom reached 10 gm., most frequently weighing from 3 to 7 gm. at the time of the animal's death. Recently, however, the yield of tumors has increased much more rapidly than was required for experimental purposes. Tumors of mice which die or which show a leucocyte count over 18,000 are never employed for re-inoculation, and these tumors have afforded ample material for the chemical investigations which are the main object of this paper.

A few analyses of human tumors have been published by Beebe, who was, however, compelled to make use of materials of various origins, which can scarcely be said to afford an accurate basis for comparison; for example, the ash of an angio-sarcoma of the leg being compared with that of a round-celled sarcoma of the shoulder. The author found that the former tumor showed signs of profound degeneration accompanied by a low potassium and a high calcium content, whilst the latter, without apparent degeneration, showed a high potassium and a low calcium content. Owing to the difficulty of comparing tumors of such diverse nature, generalizations as to age and potassium and calcium content are at least open to criticism.

The mouse tumors with which we are dealing, on the other hand, offer particularly favorable opportunities for such a comparison, being easily controlled and examined under varying conditions of virulence,

[1] No. 27. Preliminary report on the presence of an immune body in the blood of mice spontaneously recovered from cancer (adeno-carcinoma, JENSEN) and the effect of this immune serum upon growing tumors in mice infected with the same material. By HARVEY R. GAYLORD, M.D., G. H. A. CLOWES, Ph.D., and F. W. BAESLACK, B.A. Medical News, January 14, 1905.

No. 28. Preliminary communication regarding an immune body capable of inhibiting the development of cancer in mice (adeno-carcinoma, JENSEN). By G. H. A. CLOWES, Ph.D. Johns Hopkins Bulletin, Vol. XVI, No. 169, April, 1905.

No. 31. Further evidence of immunity against cancer in mice after spontaneous recovery. By G. H. A. CLOWES, Ph D., and F. W. BAESLACK, M.A.

age, and necrosis. They present, however, one objection in that they are extremely small, the errors of working being thus accentuated.

In the course of the last six months 100 tumors have been analyzed, the sodium, potassium, and calcium being determined if present. Magnesium has never been found in appreciable quantities. Several (10 to 15) of these tumors should be excluded on account of their extremely small size, 1 to 2 gm. of material yielding, for example, only 0.01 to 0.03 gm. of ash, a quantity too small to afford a reliable estimate of the potassium and calcium elements which seldom exceeded 10 per cent of the total ash. In such cases, however, it has frequently been possible to determine whether a given constituent was absent or unrecognizable, present in traces only or in considerable quantities. All the results obtained have been recorded in tabular form, including full data regarding the mouse, the tumor, the origin of the latter, etc. in order to facilitate future reference (Tables II to X).

An inspection of the tables shows that the amounts of potassium and calcium are subject to most remarkable variations; rapidly growing, large tumors showing a marked tendency to a high potassium and low calcium content, whilst the reverse is to be observed in the case of slow-growing tumors. This is so marked that in certain cases of very early tumors potassium only is to be recognized, whilst in the majority of old, necrotic tumors calcium only is present. It will be seen, furthermore, that a steady fall from a high percentage of potassium in the younger to practically none in the older tumors is accompanied by a corresponding increase from no calcium in the younger, to a high percentage of calcium in the older, necrotic tumors (Tables III, VI, VII, VIII).

To indicate clearly the relationship existing between the different groups of tumors, a small family tree containing some of the most important series in which chemical analyses have been carried out, is appended (see Table I). When a "lot" of mice is inoculated it receives an experimental number, and each individual mouse in that "lot" receives a letter and a characteristic mark for future recognition. Every few days each mouse is charted; that is to say, in the series of books provided for that purpose diagrams of the back are prepared, showing the head and the fore and hind legs, and if a tumor is developing a life-size drawing is made, showing its exact location. We are thus enabled to follow the development of the tumor with some accuracy and to form a fair estimate as to the

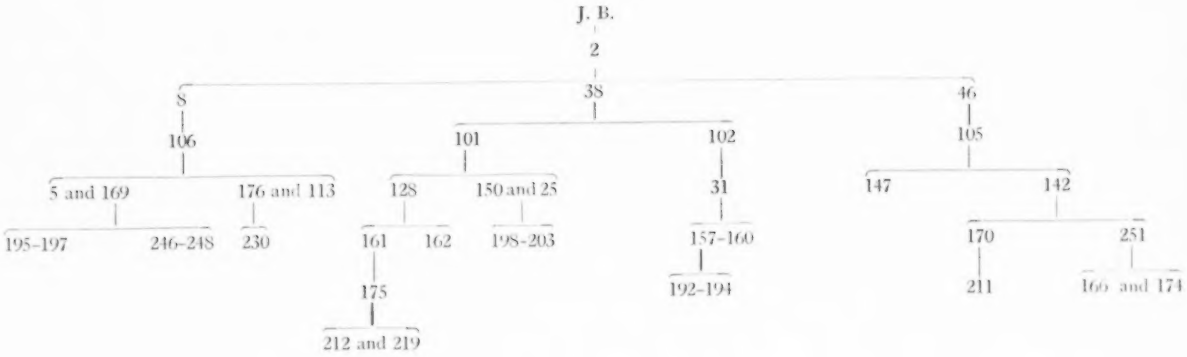
rapidity of its development. At death, the mouse plus tumor, and the tumor alone are weighed, and a portion of the latter is hardened for microscopical examination.

There are four main groups from which the tumors used for analyses have been derived, Lots 101, 102, 105, and 106. Lots 101 and 102 have an immediate common ancestor. All four lots have a common ancestor through Lot 2, in the Jensen mouse two generations previously. To facilitate an examination of the figures, the analytical results have been arranged under the four individual groups designated above, dividing the members of the group into the original series and the first, second, third, and fourth generations.

In the tables are given, first, the analytical number of the tumor for purposes of reference, followed by the experimental number and letter of the mouse. In the next column is the origin of the tumor, the immediate ancestor being first given, followed by preceding ancestors until one of the four groups, 101, 102, 105, or 106 is reached. This is followed by the date of inoculation; and the date on which the tumor had reached the size of a buckshot, having a diameter of fully 1½ to 2 m.m. The date of death or the date on which the animal was killed is then recorded. This is followed by the number of days which elapsed between the first appearance of the tumor and the date of death and the number of days from inoculation to death, the two different periods being given in order to demonstrate the relationship which obtained in some cases with one and in some cases with the other. This is followed by the weight of the mouse as a whole and the weight of the tumor, and the weight of the tumor material used for analysis in order that an estimate of the relative accuracy of such an analysis may be formed from the amount of materials employed for that purpose. The total weights of sodium, potassium, and calcium are then recorded, followed by the percentage of these elements in the original materials for analysis.

In making use of these figures it must be borne in mind that in spite of the greatest precautions observed in analytical work, considerable variations necessarily occur in the amount of water in the materials used for analysis. Consequently it is always advisable to compare the amount of one element with the amount of another rather than the absolute quantities of each present in a given weight of tumor. In addition to these tables others have been prepared supplementing the figures recorded in the general tables, in which the total weights of the three bases, sodium, potassium, and calcium, have

TABLE I.


J. B.
2
8
38
46
106
101
102
105
5 and 169
176 and 113
128
150 and 25
31
147
142
195–197
246–248
230
161
162
198–203
157–160
170
251
175
192–194
211
166 and 174
212 and 219

been added and the percentage of each determined (Tables III, VI, VII, VIII). Whilst the analytical data in the majority of cases are obtained from single tumors, in one case (Table VII) the results from all tumors falling within given age periods are averaged, a method which affords a more accurate means of estimating the percentage of potassium or calcium, since by this means a considerable error, introduced by the use of small tumors, is counteracted by the more reliable results obtained from the analyses of the larger tumors.

The earliest offshoot from the original Lot 2 was Lot 106, and although the tumors from this lot were not analyzed, the descendants may well be considered first. In the first generation eight analyses are recorded, five of them in the family of 169 and one in Lot 5 (re-inoculation), all derived from 106 A, whilst the two remaining tumors are from Lot 176, inoculated from 106 B (see Table II).

In the second generation eight analyses are recorded, three from Group 195, inoculated from 169 C; three from Groups 246, 247, and 248, inoculated at a later date with 169 P, and two of Group 230, derived from 106 through 176 B. Generally speaking, the earliest members of the series show a preponderance of potassium and the later members a preponderance of calcium, and when the immediately related tumors are considered in their respective groups, it will be seen, for example, that in the 195 group a steady fall in potassium takes place, whilst in the derivatives of 169 P there is a rise in calcium. In the 230 groups, B, which died first, has a higher potassium and lower calcium content than A. One tumor only is given in the third generation of 106, and it will be seen to correspond fairly well with the members of the series from which it is derived.

Lot 169 is dealt with independently in Table III. The total content of the three bases, sodium, potassium, and calcium, is determined, and the percentage of potassium of this total is shown for each member. It will be seen that a steady fall takes place from 40 per cent in the first of the series to 6 per cent in the last. The most marked characteristic of this group is the very high percentage of potassium and the practical absence of calcium. One tumor only, 169 G, shows a weighable amount of calcium. In noting the fact that 169 O, which died long after 169 G, shows no calcium, whilst 169 G contains a certain amount, it must be remarked that 169 O was killed whilst G died. In comparing the two tumors in the chart-book, 169 G is seen to have grown rapidly at first and to have become necrotic later, whilst 169 O grew very slowly and steadily at first, but extremely

TABLE II.

LOT 106. — FIRST, SECOND, AND THIRD GENERATIONS.

No.	Exp. no. and letter.	Origin.	Date of inoc.	Date of 1st app.	Died.	Killed.	Age of tumor in days.		Weight of mouse.	Weight of tumor.	Wt. of tumor for analysis.	Weight of Na.	Weight of K.	Weight of Ca.	Per cent of Na.	Per cent of K.	Per cent of Ca.
							1st app. to death.	Inoc. to death.									
First Generation.																	
13	169 A	106 A	Jan. 7	Jan. 17	Jan. 30	....	13	23	11.7	2.7	2.0	0.0061	0.004	trace	0.30	0.2	....
15	169 E	106 A	Jan. 7	Jan. 17	Feb. 1	....	15	25	20.5	7.6	5.0	0.0195	0.0055	trace	0.39	0.111	....
16	5	106 A	Jan. 7	Jan. 22	Feb. 3	....	12	27	29.0	9.0	6.47	0.0303	0.0051	trace	0.46	0.079	....
18	169 F	106 A	Jan. 7	Jan. 17	Feb. 6	....	20	30	12.5	3.5	3.1	0.0122	0.0041	trace	0.39	0.132	....
25	169 G	106 A	Jan. 7	Jan. 17	Feb. 20	....	34	44	18.5	4.5	4.22	0.0165	0.0032	0.0016	0.39	0.075	0.039
30	176 A	106 B	Feb. 1	Feb. 11	Mar. 13	....	30	40	22.0	7.8	6.9	0.0266	0.0076	0.0022	0.38	0.111	0.033
49	169 O	106 A	Jan. 7	Jan. 28	....	Apr. 14	76	97	29.0	6.32	3.4	0.0156	0.001	abs.	0.46	0.029	....
51	176 B	106 B	Feb. 1	Feb. 15	....	Apr. 14	58	72	33.6	7.9	4.3	0.016	0.0035	0.0018	0.37	0.082	0.042
Second Generation.																	
48	195 D	169 C–106	Feb. 24	Mar. 15	....	Apr. 14	30	49	23.5	7.0	4.1	0.0193	0.0035	abs.	0.47	0.087	....
56	195 F	169 C–106	Feb. 24	Mar. 15	Apr. 24	....	40	59	20.0	5.0	4.3	0.0241	0.0012	0.0017	0.56	1.028	0.037
59	195 G	169 C–106	Feb. 24	Mar. 30	May 9	....	41	75	....	....	10.2	0.0421	0.0025	0.003	0.41	0.025	0.029
68	230 B	176 B–106	Apr. 14	Apr. 24	....	May 23	29	39	23.8	7.6	4.9	0.02	0.0009[1]	0.0012	0.41	0.019[1]	0.024[1]
75	247 C	169 P–106	Mar. 22	Apr. 20	June 5	....	36	65	....	5.9	5.0	0.0187	0.0014	0.0009	0.37	0.028	0.018
83	246 B	169 P–106	Mar. 22	Apr. 14	....	June 5	52	75	31.6	10.5	7.1	0.027	0.0029	0.0046	0.37	0.04	0.065
88	248 D	169 P–106	Mar. 22	May 2	June 8	....	37	78	....	....	2.8	0.0097	abs	0.005	0.35	....	0.18
92	230 A	176 B–106	Apr. 14	Apr. 20	June 17	....	58	64	....	....	6.8	0.024	0.0007	0.0075	0.35	0.01	0.11
Third Generation.																	
65	232 A	195D–169C-106	Apr. 14	Apr. 22	....	May 19	27	35	27.0	6.7	4.6	0.0206	0.0018	0.0009	0.44	0.038	0.019

[1] Slight loss of K.

rapidly in the course of the twenty days immediately preceding the date on which it was killed.

We now pass on to the derivatives of Lot 101 in which the largest number of analyses are to be found (Tables IV and V). In the first generation four analyses only were completed, and unfortunately in one case the calcium was lost through accident.

From a casual inspection of the table there would appear to be absolutely no relationship between the potassium and calcium con-

TABLE III.

GROUP 169.

Number.	Experiment number and letter.	Age of tumor.		Weight of Na.	Weight of K.	Weight of Ca.	Total of bases.	Per cent of K.	Per cent of Ca.	Remarks.
		1st app. to death.	Inoc. to death.							
13	169 A	13	23	0.0061	0.0040	trace	0.0101	40	..	
15	169 E	15	25	0.0195	0.0055		0.0250	22	..	
18	169 F	20	30	0.0122	0.0041	trace	0.0163	25	..	
25	169 G	34	44	0.0165	0.0032	0.0016	0.0213	15	7.5	Grew rapidly at first, became necrotic later.
49	169 O	76	97	0.0156	0.0010	trace	0.0166	6	..	Grew slowly at first, but extremely rapidly in last 20 days, and was killed whilst still developing.

tent and the age of the tumor. This group, however, affords an interesting illustration of the possibility of rearranging the series according to the periods of development of the tumor from its first appearance to the death of the animal, instead of the full period from the date of inoculation to death. As a general rule these two periods bear a fairly constant relationship to one another, but in this particular group that is in no sense the case, the second, according to the general form of arrangement, becoming the last if we adopt the other method.

From the small supplementary Table VI it will be seen, summing the total bases and determining their respective percentages, that, grouping the tumors according to their absolute age, potassium falls steadily from 32 per cent to nil, whilst calcium rises from 9 to 15.

TABLE IV.

LOT 101. FIRST AND SECOND GENERATIONS.

No.	Exp. no. and letter.	Origin.	Date of inoc.	Date of 1st app.	Died.	Killed.	Age of tumor.		Weight of mouse.	Weight of tumor.	Wght of tumor for analysis.	Wt. of Na.	Wt. of K.	Wt. of Ca.	Per cent of Na.	Per cent of K.	Per cent of Ca.
							1st app. to death.	Inoc. to death.									
First Generation.																	
11	25	101	Dec. 5	Jan. 3	Jan. 28	....	25	44	18 7	2.8	2.4	0.0076	0.0038	0.0011	0.360	0.161	0.046
22	119	101	" 5	Dec. 31	Feb. 17	....	48	74	21.5	7 0	6.8	0.0133	abs	0.0022	0.19	....	0.032
26	152	101	" 5	Jan. 16	" 23	....	38	80	28.5	6.0	4.2	0.0113	0.0027	trace	0.126	0.065	....
38	150 C	101	" 5	Feb. 14	Mar. 30	....	44	115	28 6	8.2	5.2	0.0235	0.0030	0.0036	0.44	0.057	0.069
Second Generation.																	
19	161 O	128 -101	Dec. 21	Jan. 3	Feb. 9	....	37	50	....	7.0	4.8	0.0158	0.0039	0.0016	0.33	0.082	0.033
23	162 C	128 -101	" 21	" 10	" 17	....	38	58	....	....	4.7	0.0184	0.0017	0.0007	0.39	0.036	0.014
37	199 G	150 B-101	Feb. 28	Mar. 9	Mar. 28	....	19	28	29.0	10.5	8.8	0.0408	0.0087	0.0016	0.46	0.099	0.018
39	198 G	150 B-101	" 28	" 15	Apr. 1	....	17	32	17.0	4.0	3.0	0.0130	0.0058	0.0010	0.43	0.193	0.033
41	198 F	150 B-101	" 28	" 15	" 7	....	23	38	24.0	8.0	6.0	0.0214	0.0060	0.0014	0.36	0.100	0.027
42	201 G	150 B-101	" 28	" 9	....	Apr. 7	29	38	18.5	5.5	3.4	0.0163	0.0033	0.0009	0.47	0.096	0.026
43	198 E	150 B-101	" 28	" 6	....	" 7	32	38	18.5	4.0	2.2	0.0105	0.0032	0.0011	0.46	0.142	0.049
44	201 H	150 B-101	" 28	" 12	....	" 8	27	39	16.1	4.9	4.8	0.0178	0.0027	0.0013	0.37	0.059	0.027
52	199 D	150 B-101	" 28	" 15	Apr. 20	....	36	51	22.3	7.5	6.4	0.0308	0.0018	0.0011	0.49	0.028	0.017
53	200 K	150 B-101	" 28	" 12	" 20	....	39	51	....	4.5	4.2	0.0196	0.0004	0.0010	0.47	0.010	0.024
55	199 E	150 B-101	" 28	" 9	" 17	....	39	48	15.5	5.0	2.5	0.0189	0.0017	0.0013	0.75	0.067	0.052
57	201 K	150 B-101	" 28	" 10	" 27	....	48	58	....	....	4.8	0.0175	0.0024	0.0013	0.36	0.050	0.027
74	203 A	150 B-101	" 28	Apr. 17	June 3	....	47	95	....	....	4.1	0.0164	0.0008	0.0052	0.40	0.019	0.125
99	202 D	150 B-101	" 28	May 2	" 23	....	52	115	16.6	3.7	2.3	0.0123	abs.	0.0077	0.53	....	0.336

TABLE V.

LOT 101. THIRD AND FOURTH GENERATIONS.

Number.	Experiment number and letter.	Origin.	Date of inoc.	Date of 1st app.	Died.	Killed.	Age of tumor.		Weight of mouse.	Weight of tumor.	Weight of tumor for analysis.	Weight of Na.	Weight of K.	Weight of Ca.	Per cent of Na.	Per cent of K.	Per cent of Ca.
							1st app. to death.	Inoc. to death.									
Third Generation.																	
36	254 B	161 M-12S-101	Jan. 31	Mar. 2	....	Mar. 28	26	56	28.5	5.6	4.6	0.0186	0.0027	0.0016	0.40	0.059	0.035
40	175 D	161 M-12S-101	" 31	Feb. 17	Apr. 6	....	48	65	15.3	2.9	2.4	0.0090	0.0030	0.0030	0.37	0.125	0.025
78	228 A	201 G-150 B-101	Apr. 7	Apr. 28	June 5	....	38	59	..	..	7.6	0.0328	0.0013	trace	0.43	0.016	....
79	206 H	161S-12S-101	Mar. 3	" 6	" 5	....	60	94	29.8	6.4	3.4	0.0148	0.0018	0.0011	0.43	0.053	0.032
89	225 A	198 B-150 B-101	Apr. 7	" 13	" 13	....	61	67	..	2.0	2.0	0.0121	abs.	0.0011	0.62	....	0.056
93	237 B	199 F-150 B-101	" 19	May 17	" 20	....	34	62	..	..	6.5	0.0272	0.0022	0.0027	0.41	0.034	0.041
98	304 D	200 A-150 B-101	" 26	" 23	" 23	....	31	58	19.2	4.1	3.4	0.0159	0.0014	0.0008	0.46	0.042	0.023
Fourth Generation.																	
58	213 C	175-161-12S-101	Mar. 28	Apr. 20	May 8	....	18	41	..	..	2.9	0.0138	0.0019	0.0007	0.47	0.064	0.024
62	215 A	175 C-161 M-12S-101	" 28	" 13	" 15	....	32	48	..	..	2.9	0.0111	0.0005	0.0008	0.38	0.016	0.027
67	216 B	175 C-161 M-12S-101	" 28	" 22	....	May 23	31	56	26.0	7.5	4.6	0.0214	abs.	0.0017	0.46	....	0.036
72	110	175 C-161 M-12S-101	" 28	....	June 2	....	..	66	..	..	6.7	0.0178	"	0.0026	0.11	....	0.038
81	213 A	175 C-161 M-12S-101	" 28	Apr. 17	" 6	....	50	70	..	..	4.4	0.0159	"	0.0030	0.36	....	0.068
87	218 B	175 C-161 M-12S-101	" 28	" 27	" 9	....	43	73	..	..	5.0	0.0151	"	0.0025	0.30	....	0.049
91	116	175 C-161 M-12S-101	" 28	....	" 14	....	..	78	..	..	6.4	0.0214	"	0.0082	0.33	....	0.127

Table IV also includes the second generation of Lot 101, consisting of two tumors, 161 O and 162 C, derived from Lot 128, and twelve tumors belonging to Groups 198 to 203, all of one family, obtained by inoculation from 150 B on February 28. This group, derived from 150 B, is one of the most interesting in the entire series, fifty mice inoculated having yielded upwards of twenty large, fairly rapidly growing tumors. Whilst the twelve analyses recorded show a tendency to a steady decrease in potassium and a gradual rise in calcium, reaching very considerable proportions in the last two members of the series, there is at first sight a lack of strict uniformity in

TABLE VI.

FIRST GENERATION OF 101.

No.	Experiment number.	Age inoculated to death.	Weight Na.	Weight K.	Weight Ca.	Total bases.	K per cent.	Ca per cent.
11	25	25	0.0086	0.0038	0.0011	0.0135	28.1	8.1
26	152	38	0.0113	0.0027	Lost	0.0140	20.0	Lost
38	150	44	0.0235	0.0030	0.0036	0.0301	10.0	12.0
22	119	48	0.0133	abs.	0.0022	0.0155	..	15.0

the results. When, however, it is remembered that 201 G and 198 E were killed, which appreciably interferes with the results, and that certain of the tumors were rather small for analytical purposes, such discrepancies may readily be accounted for.

In order to reduce the experimental error to a minimum, we have grouped all the mice dying within given periods, adding together the total weight of their tumors, the total sodium, potassium, and calcium content, and determining the potassium and calcium percentage of this total. In Table VII the results are recorded, and show a most remarkably steady fall from 23.7 per cent potassium in the twenty to thirty-five day period to nothing in the one hundred and ten to one hundred and thirty-five day period, whilst the calcium content rises steadily from 4.2 per cent in the former to 38.5 in the latter periods.

Table V includes the third and fourth generation of Lot 101. In the third generation there is very little basis for comparison of the results. In the first two tumors the ratio of potassium over calcium falls slightly with increase in age. Of the four tumors derived from

150 B through the 198 to 203 group, it will be seen that the three which take from thirty to forty days for development from the date of first appearance show potassium and about equal amounts of calcium, whilst the remaining member of the group, 225 A, in which the period of development was sixty-one days, shows no potassium and the highest amount of calcium. In the fourth generation of 101, also recorded on this sheet, is the most remarkable series of individual tumors thus far encountered. All seven members of this generation were derived directly from 175 C and are, therefore, more strictly

TABLE VII.

Experiment number and letter.	Age of tumors in day periods.	Wt. of Na.	Wt. of K.	Wt. of Ca.	Total of bases.	Per cent of K.	Per cent of Ca.	Ratio of K to Ca.
199 G–198 G	20–35	0.0436	0.0145	0.0026	0.0607	23.7	4.2	5.6
198 F–201 G–198 E–201 H	35–50	0.0660	0.0152	0.0047	0.0859	17.6	5.4	3.2
199 E–201 K . . . . 199 D–200 K	50–65	0.0868	0.0063	0.0047	0.0978	6.4	4.7	1.4
None	65–80	....	....	....	....	....	....	....
203 A	80–95	0.0164	0.0008	0.0052	0.0224	3.5	23.0	0.15
None	95–110	....	....	....	....	....	....	....
202 D	110–125	0.0123	nil	0.0077	0.0200	nil	38.5	....

comparable than is generally the case. A steady fall in the potassium content is associated with an equally steady rise in the calcium content. This remarkable agreement is emphasized in the special Table VIII, in which the total bases are added together and the potassium and calcium percentage determined. It should be noted that 216 B was subjected to a treatment previous to analysis whereby a certain amount of potassium may have been lost. This tumor, although falling into the series, should therefore be excluded from any strict comparison. One member of the fifth generation of 101, No. 327 B, showed at the age of forty-seven days .117 per cent of calcium and no potassium.

Table IX gives the results of analyses carried out on the tumors derived from 102. Only six mice have been used for this purpose in the three generations, and it will be seen at a glance that the same general relationship obtains as in previous cases.

Lot 105 and its derivatives, constituting the fourth important group, are considered in Table X. Whilst five analyses of actual members of Lot 105 were carried out, two only have been included, the other three being amongst the first work of this nature carried out in the laboratory, and a certain amount of question existing as to their accuracy. It may, however, be said that in no case was potassium found to be present, although the ages of the tumors ranged from 42 to 107 days. Calcium was present in considerable quanti-

TABLE VIII.

No.	Exp. no. and letter.	Age of tumor.		Weight of Na.	Weight of K.	Weight of Ca.	Total of bases.	Per cent of K.	Per cent of Ca.
		1st app. to death	Inoc. to death.						
58	213 C	18	41	0.0138	0.0019	0.0007	0.0164	12.0	4.3
62	215 A	32	48	0.0111	0.0005	0.0008	0.0124	4.0	6.6
67	216 B	31	56	0.0214	trace	0.0017	0.0231	trace [1]	7.4
81	213 A	50	70	0.0159	trace	0.0030	0.0189	....	16.0
87	218 B	43	73	0.0151	trace	0.0025	0.0176	....	14.0
91	116	?,	78	0.0214	trace	0.0082	0.0296	....	27.4

[1] Lost.

ties in all five tumors. In the first generation two tumors analyzed show no potassium and a fair amount of calcium. In the second generation one small tumor shows potassium and a second, at 67 days, no potassium and a large content of calcium. In the third generation are five tumors, three of them derived from 251 E. 251 E was utilized originally for a series of experiments in which the virulence of the normal materials admixed with salt solution at normal temperatures, at 40° and at 45°, was compared. In this case the materials incubated fifteen minutes at 40° gave a yield of a larger number of more rapidly growing tumors than was obtained from the use of the normal material.

This work will be the subject of another paper from this laboratory, and will not therefore be dealt with at this stage, but it should simply be noted that the tumors in question made their appearance only

TABLE IX.

LOT 102 AND DERIVATIVES.

Number.	Experiment number and letter.	Origin.	Date of inoc.	Date of 1st. app.	Died.	Killed.	Age of tumor.		Weight of mouse.	Weight of tumor.	Weight of tumor for analysis.	Weight of Na.	Weight of K.	Weight of Ca.	Per cent of Na.	Per cent of K.	Per cent of Ca.
							1st app. to death.	Inoc. to death.									
First Generation.																	
14	115 C	102	Nov. 12	Nov. 30	Jan. 31	....	62	80	25.8	6.6	4.9	0.0161	0.0053	trace	0.33	0.109	....
27	129 A	102	" 12	" 30	....	Feb. 28	90	108	29.5	10.0	3.2	0.0116	0.0020	0.0030	0.35	0.061	0.092
Second Generation.																	
12	160 N	31-102	Dec. 23	Jan. 3	Jan. 28	....	25	36	11.8	2.9	2.5	0.0082	0.0034	0.0012	0.33	0.136	0.050
20	157 A	31-102	" 23	" 12	Feb. 15	....	34	54	....	4.7	3.6	0.0138	0.0016	0.0022	0.38	0.044	0.060
21	157 B	31-102	" 23	" 20	" 16	....	27	55	12.5	3.0	2.8	0.0117	0.0019	0.0018	0.41	0.067	0.063
Third Generation.																	
35	192 F	157-31-102	Feb. 22	Mar. 10	Mar. 25	....	15	31	20.0	2.5	2.0	0.0097	0.0015	0.0014	0.48	0.076	0.070

twelve days after inoculation and grew with extreme rapidity, and when examined macroscopically appeared to be necrotic, especially the earliest members of the series. This may account for the practical absence of potassium and the high calcium content of this group.

In the fourth generation of 105 are seven tumors, five of them derived from 174 G and one each from 174 B and 174 F. When this series is grouped collectively in definite periods, the same type of relationship is found to obtain as in previous cases, the only marked discrepancy being the absence of potassium in 322 C and 329 A, preceding 233 A in actual age, but it must be noted that 233 A grew rapidly at the last stage, whilst 322 C and 329 A after a rapid early development were practically stationary, were commencing to ulcerate and already becoming distinctly necrotic.

To summarize the above analytical results, it may be said that, both as a whole and also in individual groups, the most rapidly developing tumors which reach large dimensions in the course of a short time show a high potassium content and practically no calcium. The pathological examination of sections of such tumors, for example, 169 E and 5 in Table I and 199 G, 198 G and 198 F in Table IV, shows rapidly growing cancer epithelium and relatively small indications of necrosis. These tumors contained from .05 to .09 gram of potassium, an amount which is exceptional when the extremely small size of the tumors is considered. In contrast to the rapidly growing tumors, showing a high per cent of potassium, are the slow-growing tumors, many of them over one hundred days old, which generally contain over .1 per cent of calcium and no potassium. Such, for example, are 203 A and 202 D the two oldest tumors in the second generation of Lot 101, Lot 116, the oldest tumor in the fourth generation of 101, and several tumors amongst the 105 derivatives. Such tumors are not necessarily entirely necrotic. They generally contain a relatively large amount of connective tissue, but this question is one which will be the subject of further investigation in this laboratory.

A further consideration of the tables in detail shows that with tumors of relatively the same size, the age of the tumor, whether estimated on the absolute basis or simply from the time it makes its first appearance, generally bears a certain ratio to the potassium and calcium content, or the ratio of $\frac{K}{Ca}$. Thus, if the ratios of $\frac{K}{Ca}$ are taken in such a group as 198 and 203, for example, in which both elements are present in all tumors with the exception of the

TABLE X.

LOT 105 AND DERIVATIVES.

Number.	Experiment number and letter.	Origin.	Date of inoc.	Date of 1st app.	Died.	Killed.	Age of tumor.		Wt. of mouse.	Wt. of tumor.	Wt. of tumor for analysis.	Wt. of Na.	Wt. of K.	Wt. of Ca.	Per cent of Na.	Per cent of K.	Per cent of Ca.
							1st app. to death.	Inoc. to death.									
6	105	....	Oct. 10	Oct. 28	Dec. 1	....	34	52	14.7	7.5	5.2	0.0154	abs.	0.0017	0.29	....	0.032
10	105 blk.	....	" 10	" 28	Jan. 25	....	89	107	17.7	6.0	3.5	0.0134	"	0.0030	0.37	....	0.084
							First Generation.										
17	142-x ray	105	Nov. 19	Nov. 26	Feb. 6	....	72	79	20.7	4.2	2.8	0.0135	abs.	0.0017	0.48	....	0.060
24	147 C	105	" 30	Jan. 30	" 17	....	18	79	22.0	3.0	2.8	0.0104	"	0.0027	0.37	....	0.096
							Second Generation.										
31	149 D	142-105	Jan. 5	Jan. 16	Mar 13	....	56	67	23.0	5.9	5.0	0.0130	abs.	0.0058	0.25	....	0.115
							Third Generation.										
50	174 F	251 E-142-105	Mar. 17	Mar. 27	....	Apr. 14	18	28	26.1	4.1	2.3	0.0097	abs.	0.0013	0.42	....	0.056
61	174 G	251 E-142-105	" 17	Apr. 1	May 16	....	45	60	..	..	2.9	0.0131	0.0016	0.0090	0.45	0.054	0.030
73	211 C	170 F-142-105	" 15	....	June 3	....	..	80	..	..	8.1	0.0262	abs.	0.0059	0.32	....	0.072
76	166 A	251 E-142-105	" 17	Apr. 10	" 5	....	56	80	..	..	0.8	0.0026	"	0.0013	0.33	....	0.168
97	234 F	41 B-147-105	Apr. 19	May 17	" 22	....	36	64	15.1	1.3	1.3	0.0037	"	0.0033	0.28	....	0.238
							Fourth Generation.										
70	321 A	174 G-251 E-142-105	May 5	May 17	May 31	....	14	26	..	..	4.0	0.0229	0.0005	trace	0.57	0.013	....
77	321 D	174 G-251 E-142-105	" 5	" 20	June 5	....	16	31	..	..	3.7	0.0166	0.0029	0.0013	0.44	0.075	0.034
82	322 A	174 G-251 E-142-105	" 5	" 20	" 6	....	17	32	..	..	4.9	0.0215	0.0020	0.0008	0.43	0.041	0.016
90	324 A	174 G-251 E-142-105	" 5	" 23	" 15	....	23	41	..	..	5.4	0.0241	0.0012	0.0014	0.44	0.022	0.025
95	233 A	174 F-251 E-142-105	Apr. 14	Apr. 24	" 21	....	58	68	24.5	7.0	6.3	0.0257	0.0015	0.0033	0.41	0.023	0.052
96	322 C	174 G-251 E-142-105	May 5	May 27	" 22	....	26	48	17.5	5.8	5.3	0.0220	abs.	0.0019	0.41	....	0.035
101	329 A	174 B-251 E-142-105	" 16	" 29	" 28	....	30	43	19.5	6.0	5.2	0.0213	"	0.0008	0.35	....	0.015

last, it is found that $\frac{K}{Ca}$ falls gradually in value from 5.7 and 5.8 at the commencement to .15 and nil at the end of the series. This fact is most clearly illustrated in Table VII, where the ratio for the first period is 5.6, for the second period 3.2, for the third period 1.4, for the fourth period .15, and for the fifth period nothing.

The admixture of small and large tumors is misleading, large tumors having a tendency to show a higher content of potassium than is to be expected at their period of development, small tumors showing a larger content of calcium. If, however, the relative proportions of these elements are functions of the rapidity of growth, this discrepancy will readily be explained when one realizes that although two tumors may, through toxic or other influences, cause the death of mice in the same period of time, if one is twice as large as the other, its rate of development has been considerably higher. In the few cases in which discrepancies occur, a reference to the chart-books generally affords an explanation. It happened, for example, that an old tumor showed a considerable amount of potassium. In such a case it was generally observed that the tumor in question had developed slowly at first but very rapidly in the last stages. On the other hand, when a large quantity of calcium is present in a young tumor, it is generally observed that the tumor in question grew rapidly at first, then remained stationary and became necrotic previous to the death of the mouse.

In contrasting the descendants of the three main groups, 106, 101, and 105, it appears at first sight that the derivatives of 106, especially the 169 group, show a preponderance of potassium; that is to say, that at a given age and given size, and under otherwise comparable conditions, the members of this group appear to contain more potassium than do those of Group 101 and 105. Group 105, at least in the early generations, shows in the same sense a high proportion of calcium, whilst Group 101, especially through the strain of 150, shows a more even balance of these two elements than is to be found in either of the other groups.

The series of mice from 198 to 203, which, as was stated above, has yielded a higher proportion of large, rapidly growing tumors than any other series so far investigated, is most remarkable for the fact that with one exception every tumor of this series contains both potassium and calcium in appreciable quantities, the proportion of potassium to calcium obtained by adding together the total content of the twelve tumors being 3 : 2.

In considering the results of transplantation from young tumors and old tumors, we made the observation early in the work, that even rapidly growing young tumors are not suitable for this purpose; rapidly growing, partially necrotic, older tumors giving better results.

One of the most remarkable examples of a high calcium content in a young tumor is 174 F, which was killed twenty-eight days after inoculation. This tumor appeared, however, from the very first to be necrotic, and on reinoculation produced similar, although less marked results in the next generation.

In the course of these experiments six additional tumors were dried and extracted with chloroform in order to remove fatty bodies, and it is interesting to note that the potassium constituents were to a considerable extent dissolved by the extracting agent, whilst the calcium remained entirely in the residual portion.

The potassium and calcium content in its relation to virulence.— Two series of experiments were carried out. In one series twelve tumors were used both for purposes of inoculation and also for chemical analysis. In the second series of experiments tumor materials from the same source were mixed, on the one hand with a potassium-chloride and on the other hand with a calcium-chloride solution, isotonic with normal salt solution, and used subsequently for injection.

In the first series of experiments 198 F, 201 G, 198 E, and 198 B were employed on April 7 for inoculation. 198 F and 201 G, having a slightly higher proportion of potassium than 198 E, showed a higher proportion of tumors. This experiment can scarcely be considered conclusive on account of the small number of mice, five only being employed in each series. On April 14 four mice, 195 D, 169 O, 174 F, and 176 B were used for a similar purpose; but whilst the chemical analyses showed very considerable variations, the yield of tumors in each case was fairly comparable. Further experiments carried out on May 19, on 232 A, with a larger proportion of potassium than 200 L, gave four tumors as against one in 200 L, eight mice being employed in each case. These experiments are in no sense conclusive; and it will be necessary to do a considerable amount of experimental work in this direction, on account of great variations shown in the relative virulence of different tumors.

Experiments to determine the direct effects of isotonic solutions of potassium and calcium upon the development of tumors have been undertaken and are now under way. All that can be said at the

present time is that the mice inoculated and fed with potassium-holding materials appear to be more susceptible than those inoculated and fed with calcium material. No attempt has been made at present to draw conclusions based on the ionic theory, but it is interesting to note that the figures in the tables showing the ratio of potassium and calcium apply also to the ions of these elements, their relative atomic weights, 39 and 40, producing comparatively small change.

It is not our purpose to speculate at present regarding the significance of the proportions of these elements in the tumors, and we are not disposed to attribute any direct influence to the potassium as such, but consider the potassium rather as a necessary element in active growth, and the calcium a result of necrosis. It must, however, be borne in mind that calcium has been shown to exert an inhibiting influence upon potassium in a large variety of the physiological effects of this element.

Considering the great length of time through which these tumors have been transplanted, it will be realized that an immense number of subdivisions of the original cell must have taken place. It is possible that work similar to the above, carried out for a considerable period, may show evidence of an actual aging process and loss of the dividing-energy. We have already been led to suspect the existence of a form of periodicity in the virulence of the mouse tumors. There is a certain amount of evidence in the tables given above that the same may be said to exist as regards chemical composition. It is proposed to make careful observations regarding any indications of a rejuvenation process in its relationship to the varying chemical composition of the tumor series.

Summary.

It has been shown that rapidly growing, large tumors in mice contain a high percentage of potassium and little or no calcium; also that old, slowly growing, relatively necrotic tumors contain large amounts of calcium and little or no potassium.

It has been observed that in groups of comparable mice the potassium and calcium content is a function of the age and rapidity of development of the tumor, discrepancies observed being frequently attributable to more rapid growth or degenerative processes already recorded in the diagram books.

Certain groups appear to possess at given ages relatively larger proportions of potassium and others relatively larger proportions of

calcium; but the largest yield of tumors and the most rapid growth as represented by the unit of tumor produced per unit of mice in the unit of time, is apparently associated with an equilibrium in which both elements are present, the ratio averaging from 2 : 1 to 3 : 2 in favor of potassium.

OBSERVATIONS ON THE FATE OF OIL INJECTED SUBCUTANEOUSLY.

By YANDELL HENDERSON and EDWARD FRANCIS CROFUTT.

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THE question whether or not a fat introduced under the skin is absorbed and utilized is of twofold importance. Practically considered, such injections might afford a valuable method of combating cachectic conditions. Theoretically, the subject is of interest for what it may show as to the mechanism of absorption from the spaces in the subcutaneous connective tissue, and the relation of these spaces to the lymphatics. If these spaces are directly connected with lymph channels, the injected fat (at least when introduced as an emulsion) might pass readily into the lymphatics, and so into the circulation. Again, the introduction of large quantities of neutral fat either as oil or in emulsion among the subcutaneous cells, which form adipose tissue whenever the state of nutrition permits, might be expected to lead to a rapid importation of the fat into the interior of these cells.

The most recent investigations on this subject are those of Winternitz.[1] The method employed by this investigator was to inject iodized fats subcutaneously and to estimate the rate of their utilization by determining the amount of iodine appearing as iodide in the urine. The conclusion reached by Winternitz was that, while in the course of time the injected fat is completely utilized, the process is exceedingly slow. Thus an injection of 500 gm. of fat was consumed at the rate of only 2 to 5 gm. per day. The fat disappeared rapidly from the point of injection, but if the autopsy were not too long delayed, could be found scattered through the subcutaneous spaces lower down.

We believe the conclusions reached by Winternitz to be essentially correct. The investigations of v. Leube,[2] on the contrary (which

[1] Winternitz, H.: Zeitschrift für klinische Medicin, 1903, l. p. 80.

[2] Von Leube, W.: Sitzungsberichte der physikalisch-medicinischen Gesellschaft zu Würzburg, 1895, p. 5.

preceded those of Winternitz), led him, through what seems to us a misinterpretation of their results, to the opposite conclusion, — that fat introduced into the system subcutaneously is readily utilized or stored as true adipose tissue. In one of v. Leube's experiments a dog of 12.5 kilos weight was reduced in the course of two months on a diet of 250 gm. of fat free horse flesh to an emaciated condition and a weight of 10.75 kilos. During the succeeding two months 3450 gm. of melted butter were injected. No local irritation was caused; the butter rapidly disappearing from the points of injection. Von Leube notes that the weight of the animal was increased by each injection to the amount of the weight of the butter introduced. One month after the last injection the animal was killed. "On cutting through the skin of the abdomen liquid butter exuded. . . . Under the superficial muscles butter was stored, in addition to being deposited in the ordinary adipose tissue. The thickness of the layer of fat on the abdomen was 4.5 c.m., on the back 2.5 c.m. In the latter location no butter admixture could be discovered by macroscopic examination." The Reichert-Meissl figures obtained from the analyses of the fat from various localities indicated that "the belly fat consisted of practically pure butter. The largely increased fat on the back was approximately one-third butter, and the pericardial fat one-sixth. The kidney and mesenteric fat, however, did not differ appreciably from ordinary dog fat." In a second experiment a small, poorly nourished dog of 4 kilos weight received 1590 c.c. (1400 gm.) of butter under the skin. After four months on a diet of fat-free horse flesh (at first 250 gm. daily, later 200 gm. and finally only 150 gm.) the animal's weight had fallen again below 4 kilos. It was then killed and dissected. From the entire subcutaneous tissue only 3 to 4 gm. of fat could be collected, from the mesentery and from about the kidneys and heart only 2 gm. The analyses indicated an admixture of only 0.5 gm. of butter in the former, while the latter was normal dog fat. Von Leube considers this experiment to show that fat subcutaneously injected can be utilized in metabolism. We admit that, given a sufficiently lengthy period of time, such is the case. Yet it seems to us that the chief significance of this experiment is that such fat is not available for the needs of the organism in any really important amounts. Even under the extreme conditions of the experiment the injected butter disappeared slowly. In the light of our experiments we believe that what v. Leube[1] seems to

[1] Von Leube, W.: Ueber kunstliche Ernährung, in v. Leyden's Handbuch der Ernährungstherapie (2d ed.), 1903, i, p. 396.

have accepted in the case of the first dog as true adipose tissue formed from the injected butter was in reality merely connective tissue impregnated with butter. Such tissue is so similar in appearance to real adipose tissue that we were saved from confusing them only by the fact that the fat which we employed (cotton seed oil) remained fluid even when the animal under autopsy had lost its warmth. We therefore observed that even the pressure of the fingers on such pseudo-adipose tissue caused the oil to run out of it as from a sponge. Butter, on the contrary, would be solidified at room temperature, and would not then be expressible from the meshes of the connective tissue.[1]

Experiments. — In our investigations we employed cotton seed oil. This oil is easily distinguishable from dog fat by its bright yellow color, by remaining entirely fluid at ordinary temperatures, by its high iodine number (106 to 110), and by its characteristic and delicate reaction to Halphen's test.

Halphen's test is performed as follows: Carbon disulphid, containing about 1 per cent of sulphur in solution, is mixed with an equal volume of amyl alcohol. Equal volumes of this reagent and the oil under examination are mixed, and heated in a bath of boiling brine for fifteen minutes. In the presence of as little as 1 per cent of cotton seed oil an orange or red color is produced, which is characteristic.[2]

The oil which we used was a sample obtained from a dealer in oils who, in spite of the label which it bore, guaranteed it to be highly purified and unadulterated cotton seed oil. It was labelled "Huile d'Olives, Surfine, Gras, Alpes Mmes." Its reaction to Halphen's test was entirely satisfactory.

Before injection the oil was heated on a water bath while steam was bubbled through it for ten minutes. The skin at the point of injection (usually well up on the flank) was shaved, and cleansed with sublimate solution. The oil was injected warm through a large hypodermic needle connected with a burette. With the upper end of the burette was in turn connected a rubber bulb by means of which an air pressure could be exerted on the oil to hasten its otherwise slow flow through the needle.

[1] Further literature on this subject is reviewed in the paper of WINTERNITZ, and in that of v. LEUBE in v. LEYDEN'S Handbuch.

[2] HALPHEN, G.: Journal de pharmacie et de chimie, 1897, vi, p. 390. Quoted from United States Department of Agriculture, Bureau of Chemistry, 1902, Bulletin No. 65, p. 32.

For review of recent literature on this subject see EMMETT and GRINDLEY: Journal of the American Chemical Society, 1905, xxvii, p. 263.

Our observations were directed to the following points:

1. The reaction of the skin and subcutaneous tissue to the injection of fat as oil, as soda emulsion, and as acacia emulsion; and the movement of the oil under the skin.

2. The detection of the oil in the blood and lymph, as should be possible were it absorbed in appreciable quantities.

3. The influence of large quantities of oil in the subcutaneous spaces in sparing proteid metabolism.

4. The possible elimination of the oil through the skin, urine or fæces.

5. The appearance of the oil in the milk.

Before turning to our protocols we would state that it was never necessary to tie the animals during the injection. Indeed the introduction of even large quantities of oil appeared to cause no pain whatever, for it was noted that at the end of the operation the animals usually neglected to lick the affected spot. In no case was there the slightest sign of irritation or infection either at the point of injection or elsewhere. The hole made by the needle closed slowly, however, being frequently found open on the day following.

Experiment 1. January 26. Dog, 8 kilos. Injected in the right flank 15 c.c. cotton seed oil. No tumor formed, the oil spreading easily under the skin.

January 27. Point of injection examined. No secondary reaction, except a soft elastic feeling. No tumor, the oil of yesterday having disappeared; 15 c.c. injected at the same spot. A small tumor formed.

January 28. No perceptible tumor; 25 c.c. injected, forming a tumor 5 cm. in diameter.

January 29. No tumor; 50 c.c. oil injected at the same spot as heretofore. In flow unresisted, although a tumor covering a considerable area was formed.

January 30. No tumor; 85 c.c. of oil injected.

January 31. No tumor; 90 c.c. of oil injected.

February 1. Up to this date we supposed that the rapid disappearance of the oil from the point of injection was due to its absorption; and we marvelled at the rapidity of the process. On closer examination, however, a large tumor was discovered on the lower part of the abdomen, and extending along the groin and down the inside of the thigh. For the purposes of another experiment the animal was killed. On making a superficial incision over the tumor clear yellow oil flowed out freely. A large piece of the oil-infiltrated tissue was dissected out. After working it between the fingers for a few minutes there only remained a little wisp of connective tissue.

Experiment 2. — Cat. After allowing the animal to fast for three days 50 c.c. of oil were injected as in previous experiment. Next day the thoracic duct was exposed (under chloroform). The extremely small size to which the duct was contracted rendered the insertion of a cannula impossible. A couple of drops of lymph were however obtained. It was typical hunger lymph, and under the microscope was seen to be entirely free from fat globules. A sample of blood was drawn from the femoral artery and centrifugalized. No trace of oil could be detected in the serum with the naked eye or microscopically. The ether extract of this blood gave no reaction with Halphen's test. On autopsy oil was found well diffused under the skin on the side injected, below the point of introduction, but nowhere else.

Experiment 3. — Cat. After a three days' fast injected 20 c.c. of a 50 per cent soda emulsion (10 c.c. oil, 10 c.c. water, and a drop of sodium hydrate solution). Next day anæsthetized, exposed thoracic duct, and succeeded with difficulty in obtaining a few drops of lymph. It was clear, colorless, and contained no trace of oil. The blood was likewise free from oil. On autopsy it was found that the soluble constituents of the emulsion had been absorbed, leaving the injected fat diffused through the connective tissue as oil. Several cubic centimetres of the clear yellow oil were pressed out with the fingers.

Experiment 4. — Small dog, on a diet of dog biscuit. Injected daily for six successive days 50 c.c. soda emulsion (in all 300 cc. containing 150 c.c. of oil). On the seventh day the animal was killed for another purpose. On cutting through the skin of the abdomen below the point of injection 33 c.c. of oil were expressed. The connective tissue in the neighborhood was dissected out and placed in a bag of cheese cloth. When this bag was squeezed between the fingers another 35.5 c.c. of oil exuded, — or 68.5 c.c. in all.

Experiment 5. — Small dog. Injected acacia emulsion on three successive days, — in all 120 c.c. containing 48 c.c. of oil. This emulsion was taken up by the connective tissue only slowly and with difficulty. On autopsy the day after the last injection, the water of the emulsion had entirely disappeared, leaving only the clear oil diffused under the skin. Recovered 25 c.c.

Experiment 6. — Small dog. Injected clear oil for three days, in all 140 c.c. On autopsy recovered 95 c.c.

These experiments will serve to show the remarkable readiness with which the subcutaneous connective tissue imbibes oil. Notable also is the rapid spread of the oil, and the influence of gravity on its movement. Emulsions, on the contrary, are imbibed with difficulty. The soluble portion of the emulsion is absorbed. Thereafter the oil

which remains oozes through the connective tissue precisely as if it had been introduced in this form. The amounts recoverable in the neighborhood of the point of injection are practically the same under both conditions. More careful dissections and extractions of areas remote from the point of injection would, we believe, have made good nearly all of the deficiency between the oil injected and the amount recovered.[1] In no case was the slightest trace of foreign oil discoverable in the lymph or blood. This seems to indicate that even in hunger an animal can draw on the large food supply introduced under its skin to only a very small extent. Yet if the connection between the lymphatics and the subcutaneous spaces is as direct as is usually supposed, the opposite result might have been expected. These considerations, taken in connection with the experiments detailed below, seem to indicate that the oil is absorbed and transported, not in a state of emulsion, but in some form of solution.

Influence on metabolism. — In order to test the influence which oil injections might exert in assisting nutrition in an animal on an insufficient diet, and to determine also the maximum rate of utilization under extreme conditions, the following experiment was performed:

A bull-terrier bitch of 7.8 kilos weight, well nourished but not fat, was kept for two weeks on a daily diet of 200 gm. of lean beef. The animal was then placed in a cage, and fed on lean beef of weighted amount, in which the nitrogen content had been determined by the Kjeldahl method. The urine was collected each morning at the same hour by catheterization and analyzed by the Kjeldahl method. As only the general trend of the nitrogen metabolism is pertinent to our problem, these data, together with the quantities of oil injected, and other details, are summarized in the accompanying table. The body weight quoted is that on the first day of each period. The figures for nitrogen express the average daily amounts for each period.

As set forth in the table, 1080 c.c. of oil were introduced under the skin along the back. The presence of this large quantity of food material assisted the daily diet so little, however, that the animal rapidly became asthenic. Indeed, so far from diminishing the waste of tissue proteids, the days on which oil was injected showed an increase in the minus balance between nitrogen income and output.[2] To avoid the risk of the

[1] Similar observations on the rapid dissemination but slow absorption of gelatin solutions have been reported by KAPOSI: Mittheilungen aus der Grenz-Gebieten der Medizin und Chirurgie, 1905, xiii, p. 391.

[2] A similar observation has been reported by E. KOLL, who has worked on this problem under v. LEUBE and reached conclusions in conformity with the

animal dying prematurely, the daily ration was accordingly raised from 200 to 400 gm. of meat. For a time the strength of the animal was restored. Yet even on this diet the dog continued to show a steady and considerable loss of nitrogen, and before the end of the experiment had again grown very weak. At this time it presented a most peculiar appearance. At first glance the animal appeared very fat or rather bloated,

Periods. Days.	Body weight.	Meat fed.	N in meat.	N in urine.	N difference.	Remarks.
	kilos	grams	grams	grams	grams	
14	7.8	200	....	....	....	
5	7.0	200	5.3	7.7	−2.4	
6	6.8	200	5.3	8.0	−2.7	Injected 180 c.c. oil daily; total 1080 c.c.
4	7.8	400	10.6	13.6	−3.0	Extreme weakness of animal necessitated increase of diet.
5	7.7	400	10.6	12.5	−1.9	
8	7.7	400	10.6	12.0	−1.4	Animal stronger.
5	7.6	400	10.6	11.5	−0.9	
7	7.6	400	10.6	11.3	−0.7	Animal again very weak.
Final weight	7.6	....	....	....	....	

Weight of air-dry fæces for fifty-four days was 105 gm.

especially on the abdomen and legs. The back, neck, and face were, on the other hand, emaciated to an extreme degree. In this connection it is further to be noted that during the last month of the experiment, in spite of the heavy loss of nitrogen (44.8 gm.), and a considerable diminution in the subcutaneous oil, body weight was only slightly reduced. Such a retention of water by the tissues as is indicated by these observations is well recognized as a frequent occurrence in conditions of slow starvation.

On the day following the last period shown in the table, the animal was killed by bleeding. The blood was free from oil globules. The ether extract of a large quantity of serum did not yield Halphen's test.

An incision was carried from the nose along the mid-dorsal line to the tip of the tail; and from this line downward the animal was carefully skinned. The subcutaneous tissue was found to be saturated with oil, and was so far as possible removed entire. In some places nodular sacs

views of the latter. Die subcutane Fetternahrung. Habilitationsschrift, Würzburg, 1897. Quoted from v. LEYDEN'S Handbuch, *loc. cit.*, p. 397.

of cyst-like appearance were found. On incision several cubic centimetres of clear oil flowed out of these cysts, and were carefully collected. The subcutaneous tissue was then squeezed in a small hand-press, and rinsed twice in naphtha. The hypertrophied connective tissue which remained resembled coarse cotton wool, and so far as a careful examination revealed, was wholly free from true adipose tissue. Indeed in the entire subcutaneous area not a particle of adipose tissue was discoverable, except in the labia majora.

The total quantity of oil recovered amounted to 540 c.c. As the specific gravity of the injected oil was 919, the figures indicate that 452 gm. of the injected oil had disappeared in thirty-five days, or about 13 gm. per day.

When the abdomen was opened one small cyst of oil was found located extra-peritoneally on the dorsal wall. It was presumably due to an unusually deep injection. Otherwise the abdominal cavity was entirely normal in appearance. From the omentum and from about the kidneys 12 gm. of true adipose tissue were obtained. The fat of this tissue was tried out by the ordinary method. It was not obtainable by the procedure applied to the subcutaneous tissue. This fat was, we believe, wholly free from any mechanical admixture with the injected oil, yet it gave a distinct reaction (a bright cherry-red color) to Halphen's test. The fat extracted from the labia majora likewise gave a distinct reaction.

During this experiment the idea occurred to us that the disappearance of the oil from the subcutaneous spaces might be partially due to its exuding from the surface of the skin. It was noticed that the skin of the animal became distinctly greasy, even within a few days after a bath. Accordingly the skin over an oil cyst in the groin was shaved, and then wiped off daily with cotton wool moistened with naphtha. From an extract of this cotton a positive test was obtained with Halphen's reagent. There was, however, no indication that the amount of oil exuded was at all considerable.

The most striking feature in this experiment is that even after the subcutaneous spaces had remained saturated with oil for thirty-five days, no formation of true adipose tissue had occurred. Yet in the peritoneal fat at the end of this period, the peculiar constituent of cotton seed oil was distinctly recognizable. Are we to conclude from these observations, that the failure to find adipose tissue under the skin is due, not to a lack of formation of such tissue, but to its being utilized in metabolism as rapidly as formed? Or, on the other hand, are we to infer that only such fat can be stored within the living cells or utilized for the body's needs, as has been absorbed into the

blood and distributed in normal fashion throughout the system? Of the two suppositions, the latter seems to us the more probable.

The difference between the weight of oil injected and that recovered, indicates (since oil did not appear in the urine or fæces, and was exuded from the skin in small amounts only) that it was utilized to the extent of a maximum of 13 gm. per day. This figure is higher than that obtained by Winternitz, or even than that calculable

Periods (3 days).	HALPHEN'S reaction (color).	Notes.
I	None	Preliminary period.
II	None	Injected 50 c.c. oil daily; total 150 c.c.
III	None	Subsequent period.
IV	Pink	Fed 50 c.c. oil daily; total 150 c.c.

from v. Leube's experiments. Yet, taking the experiment as a whole, the significance of the figures for the fat consumption and for the nitrogen balance seems to us to be that the animal was unable to draw on the immense store of food material in its subcutaneous spaces to an extent sufficient to prevent a steady and considerable deficiency between the income of the diet and the body's expenditures.

Influence on milk. — As it is well known that the fats of the food readily appear in the milk,[1] it seemed of interest to learn whether oil introduced under the skin of an animal in lactation could be detected in the milk. To determine this point, this experiment was performed :

A small bitch in lactation was obtained. The animal weighed 5 kilos. She had five puppies which were about two weeks old at the beginning of the experiment. Three of the puppies were killed. The other two were left constantly with the bitch, except between the hours 6 A. M. to 11 A. M. daily. At the latter hour the bitch was carefully milked, — 35 c.c. to 40 c.c. being obtained daily throughout the experiment. The milk for each three-day period was united, evaporated to dryness on a water bath, and extracted with ether. The butter-fat obtained by evaporating off the ether amounted to about 10 c.c. for each period. In order to make sure of detecting the presence of cotton seed oil, in case even a trace were

[1] On this point see GOGITDSE: Zeitschrift fur Biologie, 1905, xlvi, pp. 403-419.

present, the whole of this butter-fat was employed for Halphen's test. The results of these observations are summarized in the accompanying table (p. 201). The diet of the animal throughout the experiment consisted of two dog biscuit and a small piece of lean beef daily.

In this experiment, as the table shows, cotton seed oil given by way of the mouth appeared in the milk in easily detectable amount. When, on the other hand, an equal quantity of oil was injected under the skin, neither on the days immediately following the injections, nor after a time (three to six days) sufficient to allow a wide distribution of the oil through the subcutaneous spaces, was the slightest trace of the oil detectable in the milk.

Conclusions. — Oil injected subcutaneously is readily and widely distributed through the subcutaneous spaces. Such oil is not, however, transformed *in situ* into adipose tissue. In fact, the tissues react to its presence as to any non-irritating foreign substance. In the blood, lymph, and milk it does not appear in detectable amounts. While the oil is ultimately absorbed and utilized in metabolism, the process is one of extreme slowness. Oil injections in any moderate amounts are therefore practically without nutritive value.

THE NATURE OF CHEMICAL AND ELECTRICAL STIMULATION.—II. THE TENSION COEFFICIENT OF SALTS AND THE PRECIPITATION OF COLLOIDS BY ELECTROLYTES.

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OF the properties of ions which determine their physiological action, the solution tension is the most important, for the physiological action of any ion may to a certain extent be predicted if its solution tension is known.[1] This factor of so great importance in physiology has received little attention on the part of those who have studied the precipitation of colloids. As it seemed probable that the solution tension should determine other than the physiological properties of salt solutions, I tried in this investigation to get a clear idea of how the solution tension of an ion might affect the chemical powers of a salt solution, and to see if this factor played any part in the precipitation of colloids by electrolytes, as had been suggested in an earlier paper.[2]

I. The Tension Coefficient of Salts.

In a solution of sodium chloride the sodium ion is held in the solution more firmly than is the chlorine, for to remove a sodium ion from a normal ionic solution of this salt the voltage on the electrodes must be at least 2.54 volts, so far as the sodium is concerned; whereas to remove chlorine from such a solution a voltage of 1.694 volts is sufficient, if the chlorine alone be considered. As a result of this difference in tension between the two ions there is a much greater tendency on the part of the chlorine ion to give up its electrical charge to any substance introduced into the solution and for the chlorine to pass out of solution than for the sodium ion to give up its charge. There is, in other words, more energy in the

[1] Mathews: This journal, x, 1904, p. 291; *ibid.*, xi, 1904, p. 455.

[2] Mathews: *Ibid.*, x, 1904, p. 317 (footnote).

chlorine ions than in the sodium ions; the difference in voltage in favor of the chlorine being equal to 2.54 volts — 1.694 volts, or 0.846 volts.

It is obvious that if the negative ion goes out of solution more readily than the positive, the solution as a whole has a greater tendency to give up a negative charge to anything brought within it than to give up a positive charge. Similarly, if the positive ion goes out of solution more readily than the negative, the solution as a whole has a greater tendency to give up a positive charge to anything dissolved in it than to give up a negative charge.

As the transfer of a positive charge of electricity from one substance to another oxidizes the latter, and the transfer of a negative charge reduces the substance receiving the charge, it is clear that all solutions which give up the positive charges of their ions more readily than the negative must have an absolute value as oxidizing agents; and all solutions in which the negative ions give up their charges more readily must act absolutely, though not necessarily relatively, as reducing agents; a solution in which the positive and negative ions give up their charges with equal ease is absolutely neutral, since its tendency to reduce is exactly equal to its tendency to oxidize.

The foregoing reasoning makes it clear that the absolute oxidizing or reducing power of any solution depends on the difference in solution tension of its ions and must be determined in part by the formula

$$\pi = \epsilon_a - \epsilon_c .$$

In this formula ϵ_a is the solution tension of the anion; ϵ_c is that of the cation, and π the oxidizing or reducing action in volts. If the sign of π is negative, the solution has absolutely a reducing action; if positive, it has an oxidizing action.[1]

Among the properties of salt solutions determined probably by the difference in solution tension of the ions are, then, their oxidizing or reducing powers. Thus cupric chloride is a strong oxidizing agent, owing to the fact that the copper ion has a greater tendency to give up its charge than has the chlorine ion; and a solution of sodium sulphite is a reducing agent because the sulphite ion gives up its negative charge more readily than sodium.

[1] Whether it actually oxidizes or reduces another substance will depend on the *relative* position of the other substance to it.

It is, however, clear that the difference in solution tension of the ions is not the sole factor in determining the oxidizing and reducing action of salt solutions. The total decomposition tension of the salt must also be considered, for if in a cupric chloride solution the copper ion gave up its charge the chlorine would be left unbalanced by any positively charged ion. This would cause an electrostatic stress which would act to prevent the copper passing out of solution. The tendency of any ion to go out of solution is hence modified by the total amount of energy necessary to remove the charges from both the ions. It is impossible to separate copper from a cupric salt solution by electrolysis unless energy at a sufficient potential is supplied to remove also the charge from that anion which holds its charge most weakly. The factor $\epsilon_a - \epsilon_c$ must hence be acted upon by the total decomposition tension of the salt, or $\epsilon_a + \epsilon_c$, and we obtain in this way the formula

$$\pi = \frac{\epsilon_a - \epsilon_c}{\epsilon_a + \epsilon_c}.$$

This factor π may be called the *tension coefficient* of the salt. It has already been shown in a former paper that it is the chief factor in determining the physiological action of salt solutions on motor nerves.

The numerator of the foregoing fraction, $\epsilon_a - \epsilon_c$, is a constant for any salt, irrespective of its concentration, provided the ions are of the same valence and the character of ionization does not change with a change in concentration. It will not vary greatly in any case. The denominator, however, will vary owing to the fact that the solution tension of an ion varies with its concentration. The formula therefore may be written more completely as follows: [1]

$$\pi = \frac{\left(\epsilon_a + \frac{.057}{n} \log \frac{1}{c}\right) - \left(\epsilon_c + \frac{.057}{n} \log \frac{1}{c}\right)}{\left(\epsilon_a + \frac{.057}{n} \log \frac{1}{c}\right) + \left(\epsilon_c + \frac{.057}{n} \log \frac{1}{c}\right)}.$$

In this formula ϵ_a and ϵ_c represent respectively the solution tension of the anion and cation in normal ionic solutions; c is the concentration of the ion, and n, its valence. The logarithm is the common logarithm.

By this formula the tension coefficients for normal ionic solutions

[1] See NERNST: Theoretische Chemie, 1903, p. 712.

have been calculated for the following salts (Table I). Nearly all the figures for solution tension used in this table are taken from Wilsmore's [1] calculations.

TABLE I.

TENSION COEFFICIENTS.

Salt.	ϵ of cation in volts. Normal ionic solution.	ϵ of anion in volts. Normal ionic solutions.	Tension coefficient π.	Salt.	ϵ of cation in volts. Normal ionic solution.	ϵ of anion in volts. Normal ionic solutions.	Tension coefficient π.
KI	2.92	0.797	−0.571	$CaCl_2$	2.28(?)	1.694	(?)
$NaBrO_3$	2.54	0.727(?)	−0.555	$MnCl_2$	0.798	1.694	+0.30
NaI	2.54	0.797	−0.522	$AlCl_3$	0.999	1.694	+0.233
Na_2SO_4	2.54	1.081	−0.409	$ZnCl_2$	0.493	1.694	+0.562
NaBr	2.54	1.270	−0.333	$CdCl_2$	0.143	1.694	+0.838
NaOH	2.54	1.157	−0.374	$CoCl_2$	−0.045	1.694	+1.054
KCl	2.92	1.694	−0.265	$NiCl_2$	−0.049	1.694	+1.059
NaCl	2.54	1.694	−0.1998	$PbCl_2$	−0.129	1.694	+1.173
LiCl	2.37	1.694	−0.1704	HCl	−0.277	1.694	+1.390
NH_4Cl	1.669	1.694	+0.0074	$CuCl_2$	−0.606	1.694	+2.114
$MgCl_2$	{2.26 / 1.214}?	1.694	+0.014(?)	$HgCl_2$	−1.027	1.694	+4.058
$SrCl_2$	2.49(?)	1.694	(?)	AgCl	−1.048	1.694	+4.42
$BaCl_2$	2.54(?)	1.694	(?)				

It will be noticed that the solution tension given in this table for the sulphate ion is different from the value calculated by Wilsmore. Nernst [2] gives the value 2.877 for the HSO_4 ion and 2.177 for the SO_4 ion. The behavior of the sulphate both toward the motor nerve and, as is shown farther on, toward colloidal solutions indicates, I think, that its real value is possibly less than that of chlorine. If the value of the sulphate ion is calculated on the hypothesis that when relieved of its charge the sulphate ion changes into the persulphate, or SO_4 goes over into S_2O_8, one obtains a value, by calculating the difference

[1] WILSMORE: Zeitschrift für physikalische Chemie, 1900, xxxv, p. 291; *ibid.*, 1901, xxxvi, p. 92.

[2] NERNST: Theoretische Chemie, 1903, p. 712.

in energy content of SO_4 as sulphate and SO_4 as persulphate, of 1.081 volts as the voltage necessary to relieve SO_4 of its charge and convert it to persulphate. I have used this value in my calculations with the sulphate owing to the fact that the behavior of sodium sulphate toward an electro-negative colloid is that of a salt of which the anion has about this solution tension.

II. The Relation of the Tension Coefficient to Colloids.

Does the tension coefficient of a salt play any part in the precipitation of a colloidal solution? The work of several observers has established the importance of the valence of the precipitating ion. Bechold[1] alone, so far as I know, is the only other writer who has made any suggestion of the importance of the decomposition tension of the salt, and he has not followed the suggestion further. Without entering into the general discussion of the nature of colloidal solutions, for which reference is made to the work of Bredig and other writers, it may be stated that colloidal particles react in most particulars as if they were ions of very slow velocity. Colloidal albumin forms true salts with the ions which precipitate it. Colloids behave also like ions in their reaction to the current. They migrate with or against the current depending on the nature of their charges. They have also a small osmotic pressure. There is, in fact, little apparent difference between a particle in suspension, a colloidal particle, and an ion, except a difference in size and velocity, with resulting changes in certain properties of their solutions. We may therefore treat the colloids as if they were gigantic ions of no velocity; the ions of opposite charge being the ions of water or other negative or positive ions.

In a precipitation of a colloid the actual precipitation appears to be due to the mass of the particles being increased more rapidly than their surfaces, the particles ultimately becoming so large that their surface energy is no longer sufficient to hold them in suspension and flocking occurs. We have, therefore, in the precipitation of a colloid two distinct factors to be considered. These factors are, first, the mass of the particles; and, second, the surface energy. It is, in my opinion, necessary to keep this fact in mind, for salts may conceivably produce flocking in either of two ways, *i. e.*, by altering mass, while the surface potential is left unaffected; or by reducing the surface potential.

[1] Bechold: Zeitschrift für physikalische Chemie, 1904, xlviii, p. 4.

The factors in the salts which produce flocking may be acting on either of these factors. Valence, for example, might influence flocking by altering mass, but not surface energy. For the present, leaving the matter of primary alteration in mass at one side, I wish to consider the potential of the surface energy of the particles and its relation to the tension coefficient of the salt; farther on the mass factor will be considered in connection with valence.

Adopting Bredig's idea of the nature of colloidal solutions, a colloidal particle may be pictured as a spherical ion with one or more ionic charges. In the figure a single negative charge only is represented; the oppositely charged ion is, in the case of the colloidal albumin used in these experiments, probably potassium or sodium. In Fig. 1 *A* is supposed to be a colloid with a single negative charge *b*, and the positive ion is *Na*. In *A* the surface tension represented by force *a* is constantly acting to reduce the surface, to coagulate the colloid and take it out of solution. This tendency is opposed by *b*, the force represented by the negative charge, the solution tension of the colloid, which together with the solution tension of the sodium holds the colloid in solution. Now it is clear that any increase in the value of *b* will cause *A* to increase its surface and go more completely and firmly into solution; and on the other hand anything which diminishes *b* will permit the surface tension *a* to precipitate the colloid more or less completely. The total value of *b* will vary with the ion of opposite charge, and will be greater if that ion has, like sodium, a high solution tension of its own. The actual value of *b* is given by the formula first derived, since *b* is the tension coefficient of the colloid. $b = \frac{x - \epsilon_c}{x + \epsilon_c}$, where x = solution tension of the colloid. When the cation is of high solution tension, the negative potential *b* counteracting the surface tension is high, and the hydrosol will be stable; if the cation is of low solution tension, the hydrosol will be unstable, since the amount of energy necessary to remove the colloid from solution is determined by the total decomposition tension of the colloid.

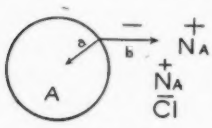

FIGURE 1.

Imagine (Fig. 1) that sodium chloride is brought into the solution. Both sodium and chlorine will have a certain tendency to deposit on the colloid and give up their charges to the colloid. If chlorine gives up its charge, it will increase the value of *b* and thus

hold the colloid in solution more firmly; if sodium deposits on the colloid, it will neutralize b more or less, reduce the tension holding the colloid in solution and precipitate it. If there is any difference between the tendencies of sodium and chlorine to deposit on the colloid, it will be obvious that the total effect of the two ions will be a preponderance of action in one direction or the other. The tendency of either ion to pass out of solution and thus to precipitate or dissolve the colloid is modified, as has already been said, by the total decomposition tension of the salt.

In the precipitation of any colloid by an alteration in the potential of its surface energy, we have therefore at least two factors to be considered: first, the tension coefficient of the colloid or $\pi_{\text{colloid}} = \frac{x - \epsilon_c}{x + \epsilon_c}$, and, second, the tension coefficient of the salt, or $\pi_{\text{salt}} = \frac{\epsilon_a - \epsilon_c}{\epsilon_a + \epsilon_c}$. For an electro-negative colloid, if π_{salt} is less positive or more negative than π_{colloid}, the colloidal solution must be rendered more stable by an addition of the salt; if π_{salt} is more positive or less negative than π_{colloid}, the stability of the colloidal solution will be lessened and, if the difference between the two is sufficiently great, precipitation must occur.

Assuming that the salt solution precipitates the colloid, it is clear that the precipitation will take one of three forms depending on the extent of the difference between π_{colloid} and π_{salt}.

1. The factor b is the factor which causes a repulsion between neighboring particles and prevents their fusion into larger aggregates. If π of the salt introduced into the colloidal solution is only slightly more positive than π_{colloid}, the repulsion between neighboring colloidal particles will be reduced, they will approach and ultimately fuse into larger aggregates without any change in their chemical composition except that a very few of the oppositely charged ions which are exerting the action will be attached to these growing aggregates. Such a condition is seen, possibly, in the gelation of gelatine where a fusion occurs of colloidal particles with only a slight admixture of the salt introduced.

2. If, however, π_{salt} is considerably greater than π_{colloid}, the foregoing process will take place, but there will be a larger proportion of the precipitating ion carried down with the aggregate in firm salt combination. If sufficient of the precipitating salt is present, a true salt of the colloid will be thrown down. This is the case,

probably, in the precipitation of colloidal egg-albumin by the heavy metals.

3. Finally, if the tension coefficient is sufficiently great and opposite in sign to that of the colloid, that is, if π_{salt} is very much greater than $\pi_{colloid}$, the precipitating ion will give up its charge to the colloid and take the charge from the colloid, in this case the albumin. One of two things may then happen. Either the albumin particle receiving the charge will be oxidized and changed chemically, if the charge is positive, thus causing an irreversible change in the colloid; or the colloidal particles relieved of their charges will unite to form a new colloid of double or greater size, just as the ions of chlorine relieved of their charges unite to form molecular chlorine. The new colloid thus formed would possibly be insoluble, and an irreversible coagulum would be formed, in which no change in composition could be detected except a change in solubility.

If the foregoing reasoning is correct, the tension coefficients of salts ought to be of great importance in determining the precipitating powers of salts, and oppositely charged ions should exert an opposite action on the colloidal solution. These conclusions were tested upon colloidal egg-albumin solution which was prepared as follows:

The egg-white was dialyzed for forty-eight hours against distilled water which was frequently changed. The solution was then diluted to twice its bulk with distilled water, and the precipitated globulins filtered out and the solution heated on the steam bath to 90° C. for ten minutes. The milky solution was then diluted to five times its bulk with distilled water. An opalescent fluid is finally obtained of slight but varying alkalinity and free from salts and globulins, in which the colloid particles are electro-negative (Hardy). Five cubic centimetres of this solution were accurately measured into test tubes, and known quantities of the salt to be tested added to different tubes until the dilution just sufficient to cause a visible precipitate in twenty hours was reached. As the degree of alkalinity of the colloid makes some difference in its stability, only solutions of the same alkalinity can be used for comparative work. While the end point is not very sharp, the results obtained on different lots of albumin were concordant. For example, three independent determinations on different solutions of egg-albumin gave 0.012 N; 0.014 N; 0.015 N as the weakest precipitating concentration of calcium chloride.

III. Is the Solution Tension or the Valence of a Precipitating Ion the more Important Factor in the Precipitation of an Electro-Negative Colloid?

To answer this question, I used at the outset unboiled egg-albumin which had been dialyzed for two days, diluted to three times its

TABLE II.

Unboiled, Dialyzed Egg-Albumin

Salt.	π Normal solution.	Concentrations of salt.	Effect on colloid.
KCl	−0.265	2 *n* and less	Clarifies
NaCl	−0.199	3 *n* " "	"
LiCl	−0.1704	4 *n* " "	"
NH_4Cl	....	2 *n* " "	"
$BaCl_2$	....	$\frac{n}{300}$; $\frac{n}{150}$; $\frac{n}{3}$	"
$SrCl_2$	....	" " "	"
$MgCl_2$	+0.014	" " $\frac{n}{1.5}$	"
$CaCl_2$	....	" " $\frac{n}{25}$	"
$MnCl_2$	+0.30	0.0033 *n*	Precipitates slowly
$AlCl_3$	+0.258	0.0033 *n*	" at once
$ZnCl_2$	+0.562	0.0033 *n*	" "
$CdCl_2$	+0.838	0.0033 *n*	" "
$NiCl_2$	+1.059	0.0033 *n*	" "
$CoCl_2$	+1.054	0.0033 *n*	" "
HCl	+1.390	0.0005 *n*	" slowly
$PbCl_2$	+1.421	0.0033 *n*	" at once
$FeCl_3$	....	0.0016 *n*	" "
$CuCl_2$	+2.114	0.0033 *n*	" "
$AgNO_3$	+4.42 (?)	0.0016 *n*	" "

volume with distilled water and the precipitated globulin filtered out. The solution was a little opalescent.

It is apparent from this experiment (Table II) that it is the solution

tension of the cation, or, more properly speaking, the tension coefficient of the salt, which determines whether a salt will precipitate unboiled egg-albumin or not. Valence as such evidently does not of itself determine whether salts precipitate, for magnesium, calcium, and barium salts, although containing bivalent cations, do not precipitate, but on the contrary they render the solution clearer. The tension coefficient separates the salts sharply into two classes, those which have a tension coefficient for their normal ionic solutions less than +0.3 all render the solution clearer, and in no concentration used did they show any sign of precipitation; while those with a tension coefficient +0.3 or greater precipitate, and in general they precipitate more promptly, the higher their tension coefficients. Contrast, for example, manganese chloride and lead chloride: the former at a concentration 0.0033 N precipitates very slowly; while the latter at even greater dilutions precipitates promptly and completely. No attempt was made in this experiment to determine the weakest concentration which would precipitate.

I next tested the boiled colloidal egg-albumin prepared in the manner described. When egg-albumin is boiled its particles increase apparently in size, and it may be anticipated that such a coarse colloidal solution will have a lower tension coefficient than the unboiled, and consequently will be precipitated by salts which did not precipitate the other. This was found to be the case, and the results given in Table III were obtained.

This experiment shows that all salts having a tension coefficient greater than 0 precipitate, and in general they precipitate more powerfully the greater their tension coefficients. Contrast magnesium chloride and silver nitrate. All salts having a tension coefficient less than −0.1 do not precipitate in any concentration I have tried, *i. e.*, as high as a 3 N solution for sodium chloride. Instead of precipitating, these salts dissolve the colloid and render it more stable, as will be shown farther on.

There are certain irregularities in the table which deserve notice, such, for example, as the greater precipitating power of zinc chloride than cobalt chloride. These irregularities, to be ascribed in part to hydrolytic dissociation, are discussed on page 226. The order of precipitating action of the alkaline earths, as may be seen from the table, is magnesium, strontium, barium, and calcium. This is the order the salts take in their toxic action also. The solution tension of these ions is uncertain, except that they come between 1.044 and

2.54. It is possible that their solution tensions may also have the same order, calcium having the lowest solution tension, although, as computed from the heat of formation, calcium and magnesium have the same tension. The tension coefficient of the colloid in a solution of this alkalinity evidently lies about 0, but is probably a little less than this and in the neighborhood of —0.1.

TABLE III.

BOILED, DIALYZED, ALKALINE EGG-ALBUMIN. (ELECTRO-NEGATIVE.)

Salt.	π for normal ionic solutions.	Weakest concentration which precipitates in 20 hours.	Salt.	π for normal ionic solutions.	Weakest concentration which precipitates in 20 hours.
KI	−0.571	No precipitation.	$AlCl_3$	+0.233	0.00072 *n*
NaBr	−0.333	"	$MnCl_2$	+0.30	0.0039 *n*
Na_2SO_4	−0.409	"	$ZnCl_2$	+0.562	0.00072 *n*
KCl	−0.265	"	$CdCl_2$	+0.838	0.00066 *n*
NaCl	−0.1998	"	$CoCl_2$	+1.054	0.0021 *n*
LiCl	−0.1704	"	$NiCl_2$	+1.059	0.0013 *n*
NH_4Cl	+0.0074	"	HCl	+1.390	0.00072 *n*
			$PbCl_2$	+1.173	0.00048 *n*
$MgCl_2$	+0.014	0.033 *n*	$CuCl_2$	+2.114	0.00036 *n*
$SrCl_2$	....	0.025 *n*	$HgCl_2$	+4.058	0.00048 *n*
$BaCl_2$	....	0.019 *n*	$AgNO_3$	+4.42(?)	0.00039 *n*
$CaCl_2$	....	0.012 *n*			

The relation of the salts to electro-positive albumin confirms these results. To make the albumin electro-positive it is necessary to make the solution acid. I accordingly added just sufficient acid (hydrochloric) to precipitate it, and then as much acid more as was required to precipitate. The solution was thus made as acid as it had been alkaline. We should now expect that since the sign of the colloid has been reversed, all the salts lying at the upper end of the table of tension coefficients should precipitate the colloid, and those farther down should not. It was impossible to predict the exact point of separation of the salts into the two classes, owing to the fact that the tension coefficient of the colloid in acid solution was unknown.

Experiment (Table IV) confirms this expectation. This table shows that all salts of monovalent acids having a tension coefficient less than +1.059 now precipitate, and they precipitate more strongly the greater the difference between their tension coefficients and this number. Thus potassium iodide precipitates more powerfully than potassium bromide, and this more powerfully than the chloride. Sodium chloride is more powerful than lithium chloride or ammonium

TABLE IV.

ELECTRO-POSITIVE COLLOID. ACID EGG-ALBUMIN.

Salt.	π	Limiting precipitating concentrations.	Salt.	π	Limiting precipitating concentrations.
KI	−0.571	0.0091 *n*	$MgCl_2$	+0.066(?)	0.038 *n*
$NaBrO_3$	−0.555	0.0091 *n*	$SrCl_2$	?	0.047 *n*
Na_2SO_4	−0.(4091)?	0.0029 *n*	$BaCl_2$	?	0.050 *n*
NaBr	−0.333	0.0148 *n*	$CaCl_2$	?	0.057 *n*
KCl	−0.2655	0.039 *n*	$MnCl_2$	+0.30	0.057 *n*
NaCl	−0.1998	0.023 *n*	$BeCl_2$	?	0.11 *n*
LiCl	−0.1704	0.0344 *n*	$ZnCl_2$	+0.562	0.056 *n*
RbCl	−0.2655	0.038 *n*	$CoSO_4$	?	0.02 *n*
Na valerianate	(?)	0.0025 *n*			
Na butyrate	?	0.0025 *n*	$NiCl_2$	+1.059	∞ [1]
NH_4Cl	+0.0074	0.034 *n*	$HgCl_2$	+4.058	∞

[1] The ∞ sign means that no precipitation took place in any concentration tested. I have not tried saturated solutions.

chloride. Calcium chloride requires a greater concentration to precipitate than barium chloride; and this than strontium or magnesium. On the other hand, nickel chloride, hydrochloric acid, mercuric chloride, and cupric chloride will not precipitate in any concentration I have tried. No salt having a tension coefficient greater than +1 precipitated unless the valence of its anion was greater than 1.

There is, however, one point of difference between these results and those obtained with the electro-negative colloid. With the

electro-negative colloid the salts which do not precipitate have a dissolving action on the colloid and render it more stable, so that larger amounts of the precipitating salts are necessary to precipitate it.

With the electro-positive colloid this is not always the case. While nickel, copper, and mercury chlorides will not precipitate this colloid, except possibly in very strong concentration, they nevertheless render it less stable instead of more stable. This may be shown by determining the minimum amount of sodium bromide necessary to precipitate, and comparing it with the minimum amount necessary to precipitate after mercuric chloride is added. It was found that the addition of mercuric chloride made it possible to precipitate with weaker solutions of the bromide instead of the reverse, as I had expected. The fact that these salts do not render the colloid more stable, as the theory demands, may possibly be due to the cations of the heavy metals forming complex cations with the colloid similar to the union of ammonia and platinic chlorides, and to these complex cations having a lower tension coefficient than the colloid itself, and so being more easily precipitated. Sufficiently strong acid also will precipitate. This is contrary to the hypothesis, unless a chemical change occur in the albumin.

This experiment confirms, with the exceptions just mentioned, the hypothesis of the importance of the tension coefficient of the salt in the precipitation of both positive and negative, boiled and unboiled egg-albumin.

IV. The Effect of Changing the Tension Coefficient of the Colloid.

A necessary conclusion of the premises is that if the tension coefficient of the colloid could be changed, the line of division between dissolving and precipitating salts should shift upward or downward and salts which formerly dissolved should be made to precipitate. This change in the tension coefficient of the colloid can be brought about by boiling the egg-albumin. By this means the salts of the alkaline earths are made to precipitate where formerly they dissolved. The change can also be produced in the boiled egg-albumin by reducing the amount of hydroxyl ions in the solution. If these be diminished, the stability of the colloid is diminished, since the effect of sodium hydrate with its negative tension coefficient is to hold the colloid in

solution by increasing b (Fig. 1). By greatly reducing the amount of hydroxyl in the solution, the tension coefficient of the hydrate is raised, and the value of b is thereby reduced; and *vice versa*. If the value of b is sufficiently reduced, salts which formerly dissolved ought now to precipitate and those which precipitated before ought to precipitate in greater dilution. The addition of more hydrate should have a reverse effect. Results confirmed the hypothesis. It is possible, as is shown in Table V, by the addition of acid, so that

TABLE V.

Showing effect of addition of acid and alkali on concentration of salt necessary to precipitate. (– means no precipitate; + means precipitate. The figures give the weakest precipitating concentrations in terms of normal solution.)

Salt.	C after addition of NaOH.	C before addition of NaOH.	C after partial neutralization by HCl.	Salt.	C after addition of NaOH.	C before addition of NaOH.	C after partial neutralization by HCl.
KI	–	–	Dissolv'd ppt.	LiCl	–	–	+; <0.01
$NaBrO_3$	–	–	"	NH_4Cl	–	–	+; <0.01
NaBr	–	–	"	$MgCl_2$	+; 0.11	+; 0.03	+; 0.0014
KCl	–	–	"	$SrCl_2$	+; 0.07	+; 0.047	+; <0.007
Na_2SO_4	–	–	"	$CaCl_2$	+; 0.024	+; 0.012	+; <0.007
NaCl	–	–	+; 0.01	$NiCl_2$	+; (?)	+; 0.0021	+; <0.00076

the hydroxyl ions are greatly reduced in numbers, to render the colloid so unstable that it is precipitated by ammonium chloride, lithium chloride, and sodium chloride, and dissolved by all salts with a lower tension coefficient. One can even render it so unstable that potassium chloride precipitates and salts having a tension coefficient less than —0.3 will dissolve. We are thus able in fact to make a colloid which behaves remarkably like many kinds of protoplasm toward which potassium chloride and sodium chloride have quite different actions. This is a matter which will be considered in another paper.[1]

[1] These results are closely similar to observations of POSTERNAK (Annales de l'Institut Pasteur, xv, 1901) upon the albumin extracted from Picea excelsa. In alkaline solutions sodium chloride and sodium bromide precipitated, while the nitrate and iodide did not precipitate.

It is clear from these results that some colloids may have tension coefficients so low that practically all salts will precipitate them. This appears to be the case, for example, with colloidal arsenious sulphide, which is precipitated by so many electrolytes. Toward such colloids the rôle of the tension coefficient will be far harder to detect than is the case with the albumin, but its value may none the less be seen in the work of most observers who have studied these colloids. Many of them have recorded differences between the precipitating power of the chlorides and iodides, for example.

V. The Relation of Ionic Valence to Precipitating Power.

All observers agree that the valence of the ion of opposite charge to the colloid is of great importance in determining its precipitating power. Thus the observations of Spring, Picton and Linder and Hardy and others have shown that salts of which this ion is bivalent will precipitate in concentrations about $\frac{1}{30}$ as great as salts of which the precipitating ion is monovalent; and that salts of which the precipitating ion is trivalent will precipitate in concentrations about $\frac{1}{1620}$ as strong as the monovalent.

On the other hand the valence of the ion of the same sign as the colloid appears to exert no influence, for cobalt chloride and cobalt sulphate will precipitate an electro-negative colloid in approximately the same dilutions.

The fact that the valence of the ion of the same sign as the colloid is unimportant has led to the conclusion that this ion exerts no action, a conclusion which is at variance with the theoretical basis of the present investigation. Pauli alone has suggested and shown that the positive and negative ions have opposite actions. Inasmuch as the opposite action of the two ions comes out most clearly in the physiological activities of the salts, I have given special consideration to this matter.

a. The importance of the ion of the same sign as the colloid. — As has been said, nearly all observers have attributed no action at all to the ion of the same sign as the colloid. Thus in a recent paper Freundlich[1] concludes that this ion is of no importance at all. Only Pauli has emphasized the fact from his experiments on egg-albumin, that the ion of the same sign as the colloid has an opposite or dissolving action on the colloid. While the opinion is widespread that

[1] Freundlich: Zeitschrift für physikalische Chemie, 1903, xliv, p. 129.

this ion has no action, indications are not lacking that some importance must be attributed to it, since nearly all observers have recorded differences in the precipitating power of the chlorides, bromides, and iodides of the same metals. In all cases to precipitate an electro-negative colloid more of the iodide is required than of the chloride or bromide. These differences, as Posternak [1] pointed out, cannot be referred to differences in velocity of the ions since the velocities of these three ions are almost identical.

My own results show that the ion of the same sign as the colloid has an important action and that it has a dissolving action on the colloid. This may be easily seen in the case of electro-negative albumin by a comparison of the minimum precipitating concentrations of the chlorides, bromides, and iodides of the alkaline earths. In this case the precipitating ion is the metal. The following results were obtained for the minimum precipitating concentrations:

$CaCl_2$, .018 N; $SrCl_2$, .022 N; $BaCl_2$, .010 N
$CaBr_2$, .0196 N; $SrBr_2$, .024 N; $BaBr_2$, .013 N
CaI_2, .031 N; SrI_2, .050 N; BaI_2, .015 N

In each of these cases more of the iodide is required than of the chloride. Inasmuch as the number of ions is probably the same in the two cases, the greater concentration required for the iodide can only be attributed to the antagonistic action of the anion. The dissolving power of the anion is evidently determined by, or inversely proportional to, the solution tension, since the solution tensions are as follows:

Cl = 1.694
Br = 1.27
I = 0.797

The results for the barium salts in the foregoing experiments were obtained on a different colloidal solution, less alkaline than that used for the calcium and strontium.

With electro-positive egg-albumin similar results were obtained. In these chlorides the precipitating ion is the chlorine. The per cent of dissociation in the various monovalent chlorides is practically the same, yet there is a considerable difference between their precipitating powers. This difference is marked between ammonium and sodium chlorides.

[1] POSTERNAK: Annales de l'Institut Pasteur, 1901, xv, p. 85.

Salt.	Minimum concentration which precipitates.
NaCl	0.020 M
RbCl	0.029 "
KCl	0.029 "
NH_4Cl	0.033 "
LiCl	0.038 "
CsCl	0.040 "
$\frac{1}{2}$ $MgCl_2$	0.029 "
$\frac{1}{2}$ $SrCl_2$	0.030 "
$\frac{1}{2}$ $BaCl_2$	0.033 "
$\frac{1}{2}$ $CaCl_2$	0.044 "
$\frac{1}{2}$ $BeCl_2$	0.110 "

The dissolving action of the ion of the same sign as the colloid may be clearly shown, also, by partially precipitating an electro-negative colloid with hydrochloric acid. Such a partially precipitated colloid is completely precipitated by sodium chloride by means of the sodium ion, while the bromide, the sulphate, the bromate, and the iodide dissolve the precipitate already formed and render the solution stable and clear. This may be seen in the following experiments.

In the first experiment sufficient acid was added to cause a permanent precipitate. The colloid did not settle out completely in forty-eight hours, but remained permanently partially precipitated. Each test-tube contained 5 c.c. of this mixture of solution and precipitate. The amount of each salt solution added, the strength of the solution, and the result are as follows:

1. NaBr 4 *n* . . .	0.1 c.c.	Precipitates.
	0.2 c.c.	Partial clearing.
	0.3 c.c.	" "
	0.4 c.c.	Good clearing.
	0.5 c.c.	Clarifies.
2. NaCl 2 *n* . . .	0.1 c.c.	Precipitates.
	0.2 c.c.	"
	0.3 c.c.	"
	0.4 c.c.	"
	5.0 c.c.	"
3. KCl *n*	0.2 c.c.	"
	0.4 c.c.	"
	0.6 c.c.	"
	0.8 c.c.	"

4. RbCl n . . .	0.1 c.c.	Precipitates
	0.3 c.c.	"
	0.6 c.c.	"
	1.0 c.c.	"
5. $NaBrO_3$ 2 n . .	0.1 c.c.	"
	0.2 c.c.	Slight clearing.
	0.3 c.c.	" "
	0.4 c.c.	Clears slowly.
	0.5 c.c.	Clears quickly.
6. Na_2So_4 m . .	0.1 c.c.	Precipitates.
	0.2 c.c.	"
	0.3 c.c.	Slight clearing.
	0.5 c.c.	Clears slowly.
	1.0 c.c.	Clears about like NaBr.
7. KNO_3 2 n . .	0.1 c.c.	Precipitates.
	0.2 c.c.	"
	0.3 c.c.	Slight clearing.
	0.4 c.c.	Clears better than KCl.
8. Na_2SO_3 $\frac{m}{10}$ . .	0.1 c.c.	Clears at once.
	0.3 c.c.	" " "
$\frac{m}{40}$ . .	0.1 c.c.	" " "
9. NaOH $\frac{n}{40}$ . .	0.5 c.c.	Not clear.
	0.1 c.c.	Clears slowly.
10. LiCl 4 n . . .	0.1 c.c.	Precipitates.
	0.3 c.c.	"
	5.0 c.c.	"
11. CaI_2 $\frac{m}{66}$ . . .	0.1 c.c.	Complete and instant precipitation.
12. KI 4 m . . .	0.1 c.c.	Precipitates.
	0.2 c.c.	Partially clear.
	0.3 c.c.	Clear.
13. K Formate $\frac{m}{2}$.	0.1 c.c.	Precipitates.
	0.3 c.c.	"
	0.6 c.c.	Partial clearing.
	1.0 c.c.	Nearly clear.
14. Na Butyrate $\frac{n}{8}$.	0.1 c.c.	Clear.
	0.5 c.c.	Clears at once.
	0.25 c.c.	" " "

Experiment 30. — Dissolving action of anions on alkaline egg-albumin. Coagulated egg-albumin. 5 c.c just precipitated by 0.5 c.c. $\frac{n}{50}$ HCl. To 100 c.c. of the alkaline solution, 9 c.c. of $\frac{n}{50}$ HCl were added. This produced a marked turbidity, but no precipitate in five hours. 5 c.c. of this solution was placed in each test tube and the following salts added.

		Result.
1.	0.10 c.c. Na_2SO_4 *m.*	Clarifies. Remains clear > 24 hours.
2.	0.20 c.c. "	" " " " " "
3.	0.10 c.c. KCl *n.*	" " " " " "
4.	0.20 c.c. " "	" " " " " "
5.	0.30 c.c. " "	" " " " " "
6.	0.10 c.c. RbCl *n.*	No change.
7.	0.20 c.c. " "	Not clear. Becomes more turbid.
8.	0.30 c.c. " "	" " Turbid. Precipitates.
9.	0.40 c.c. " "	Coagulates rapidly.
10.	0.05 c.c. LiCl 2 *n.*	" "
11.	0.10 c.c. " "	" "
12.	0.20 c.c. " "	" "
13.	0.05 c.c. NH_4Cl 4 *n.*	Coagulates partially.
14.	1.00 c.c. " "	Clarifies at first. Turbid after 1 hour.
15.	0.10 c.c. " "	Gradual turbidity.
16.	0.20 c.c. " "	Coagulates slowly.
17.	0.10 c.c. RbCl *n.*	No clarification.
18.	1.00 c.c. Na_2SO_4 $\frac{n}{5}$.	Very clear. Remains clear.
19.	Control.	Remains turbid.
20.	0.10 c.c. NaI *n.*	Clears at once, better than KCl.
21.	0.05 c.c. " "	Slight clearing.
22.	0.15 c.c. " "	Clear. Remains clear.
23.	0.20 c.c. " "	" " "
24.	1.00 c.c. " "	Very clear. Best of all.
25.	0.05 c.c. NaBr 3.9 *m.*	Clears slowly.
26.	0.10 c.c. " "	" "
27.	0.15 c.c. " "	Clarifies well.
28.	0.10 c.c. " "	" "
29.	0.05 c.c. " "	Apparently hastens coagulation.
30.	0.20 c.c. " "	Clarifies.
31.	0.50 c.c. " "	Clears very rapidly.
32.	Control.	Gradual increase in turbidity.
33.	0.05 c.c. NaCl 3 *n.*	Hastens coagulation.
34.	0.10 c.c. " "	" "
35.	0.20 c.c. " "	Heavy precipitate.
36.	0.40 c.c. " "	Clears slightly. Then precipitates.

In Experiment 30 it will be seen that sodium bromide, sodium iodide, potassium chloride, sodium sulphate, and sodium sulphite all clarify the egg-albumin, while rubidium chloride, sodium chloride, lithium chloride, and ammonium chloride precipitate the solution.

These results become clearer if the tension coefficients of the salts are written, as in Table VI.

To one tube I added hydrochloric acid until the reaction was faintly acid to litmus, and nearly the whole of the albumin had been precipitated. To this solution and precipitate I added 1 c. c. of a 4 *n* NaBr solution. The precipitate was dissolved and the solution was partially clarified. To reprecipitate it the addition of several drops of HCl $\frac{n}{55}$ was necessary. The sodium bromide used was entirely neutral to litmus and phenolphthalein.

Not only does the sodium bromide render the solution more stable toward acid, but also toward all other precipitating salts. For ex-

TABLE VI.

Salt.	Tension coefficient.	Action on colloid.	Salt.	Tension coefficient.	Action on colloid.
Na_2SO_3	(?)	Strongly clarifies.	KCl	−0.2657	Weak clarification.
NaOH	−0.374(?)	" "	RbCl	−0.2(?)	Weak precipitant.
KI	−0.577	" "	NaCl	−0.1979	Precipitates.
NaI	−0.522	" "	LiCl	−0.1663	Strong precipitant.
NaBr	−0.333	" "	NH_4Cl	+0.0074	" "
Na_2SO_4	−0.409	Clarifies.			

ample, 0.1 c.c. of a $\frac{m}{3.3}$ solution of calcium chloride caused instant coagulation of 5 c.c. of the colloidal (negative) albumin. If, however, 0.5 c.c. of NaBr 4 *n* was added first, the calcium chloride no longer caused a precipitate in this concentration.

From these experiments there is no mistaking the general conclusion, *i. e., the ion of the same sign as the colloid is not inert, as has been supposed, but exerts a dissolving action on the colloid. The power of this ion is dependent on its solution tension.*

The dissolving action of the sodium sulphate, which is about equal to that of sodium bromide, would indicate that the sulphate ion has a solution tension about that calculated.

As regards the valence of the ion of the same sign, my results agree with those of others that this is of no importance in the ion's action. Cobalt chloride and cobalt sulphate each precipitated electronegative albumin in approximately $\frac{m}{333}$ concentration. A similar re-

sult was obtained with copper acetate and copper sulphate. The fact that the valence of this ion is of no importance is of great interest in that it aids in understanding the importance of the valence of the ion of opposite sign.

b. The importance of the valence of the ion of opposite sign to the colloid. — My results confirm the work of others, that the valence of this ion is one of the most important factors in determining its action. Sodium sulphate, although its tension coefficient is only a little different from that of sodium bromide, precipitates in much greater dilution than the bromide. The limiting molecular concentration is about one-tenth that of sodium chloride. While the valence is thus shown to be of great importance, the variation from the figures calculated by Whetham,[1] is very great. According to Whetham's formula, the sulphate should precipitate in one-thirtieth the concentration of the chloride. The variation is so great as to indicate that Whetham's reasoning is incorrect or else other factors come into play with this colloid at least.

The following summary gives the concentrations of salts of the different bivalent anions used which would just precipitate:

Na_2SO_4	$\frac{m}{680}$; $\frac{m}{656}$
Na_2SO_3	$\frac{m}{780}$; $\frac{m}{800}$
$CoSO_4$	$\frac{m}{300}$
$CuSO_4$	$\frac{m}{177.6}$; $\frac{m}{176}$
Na Succinate . . .	$\frac{m}{468}$ — $\frac{m}{816}$
$K_2C_2O_4$	$\frac{m}{536}$ — $\frac{m}{780}$
$K_2Cr_2O_7$	$\frac{m}{1198}$

The only explanation thus far offered for the importance of the valence of the ion of opposite sign is that of Whetham. According to this explanation the greater precipitating power of a polyvalent ion depends on the fact that there is a far greater chance of two or three charges being near a colloidal particle at one instant if borne on one ion than if carried on two or three separate ions. I am of the opinion that Whetham's explanation is not correct, or at least that it does not explain all the facts. Were that explanation right, by the same reasoning a polyvalent ion of the same sign as the colloid ought to be greatly more powerful in dissolving the colloid than a univalent, which is not the case. It seems more probable to me that the difference in the importance of the valence of the ions of the same

[1] WHETHAM: See HARDY, Journal of physiology, 1899, xxiv, p. 301.

and opposite signs depends upon the fact that the ions of the opposite sign unite with the colloidal particles, whereas the ions of the same sign do not. It seems to me probable that the greater efficiency of the ions of opposite sign, if polyvalent, may be due not to their action on the surface energy of the colloidal particles, but to their action on the mass of the particles. For they are able because they are polyvalent to form larger molecular aggregates than the monovalent ions can form. The bivalent ions may form aggregates of this sort: —colloid—sulphate—colloid—sulphate—colloid—sulphate—, just as such aggregates are probably formed in magnesium sulphate solution. Polyvalent ions, therefore, not only precipitate by increasing the size of the colloidal aggregates by altering their surface energy through the tension coefficient of the salt, but by means of their valence they can directly increase the mass of the aggregates. To precipitate a colloid the mass of the particles must be increased by the fusion of several particles or the surface energy diminished, or both these processes must occur. As a polyvalent ion can increase mass in both ways, its valence as well as its tension coefficient comes into play. Inasmuch as a change in surface energy only is involved in the dissolving of a colloid, the valence of the ion of the same sign as the colloid is of no importance.

It is, I think, necessary to assume for the precipitating ion that the total surface of this salt-colloid aggregate is less than the surface of the aggregates not united. Otherwise the relation of mass to surface would remain unaltered by the fact of valence; but it is not unlikely that a true fusion would occur and the surface thus be greatly reduced.

If the valence of the precipitating ion is greater than unity, the result is that the ion has a precipitating power much greater than a univalent ion of the same solution tension. The effect of having a valence greater than unity is the same as if the solution tension of the ion had been reduced. Exactly in what form this correction is to be applied to the formula of the tension coefficient is not entirely clear to me; nor is it possible to foresee to what numerical extent the activity of the ion will be increased by its valence. The solution tensions of the alkaline earth metals are so uncertain that I am unable from my results to compute this correction. It will possibly take the form $\epsilon_c - A\,(n-1)$, in which ϵ_c is the solution tension of the precipitating ion, A some positive number, and n the valence of this ion. The formula becomes then:

$$\pi = \frac{\epsilon_a - [\epsilon_c - A(n-1)]}{\epsilon_a + \epsilon_c}.$$

If we assume that the sulphate ion has a solution tension of 1.08 volts, A would be nearly 3; if the sulphate is 2.17, A would be 4.

c. The velocity of the ions. — Several observers have suggested that the velocity of the ions, particularly those of the same sign as the colloid, is of importance in determining the action of the salt. My own observations do not permit a decisive answer to this question, although in their physiological activity there is hardly any doubt but that ionic velocity is of great importance. Some of the observations may, however, be interpreted in this way. For example, potassium is generally credited with a higher solution tension than sodium. It should therefore precipitate an electro-negative colloid less readily than sodium chloride. The results confirmed this hypothesis, for it is possible to make a colloidal solution which is precipitated by sodium chloride and dissolved by potassium chloride (see experiment, page 221). But while this result fully confirmed expectations and indicated that toward a colloid of opposite sign velocity is of small importance, a different result was obtained toward an electro-positive colloid. Toward such a colloid potassium chloride is slightly less efficient as a precipitant than sodium chloride, whereas were the tension coefficient the only factor it ought to be more efficient than the chloride. Since this result has been obtained by others, it appears not improbable that the velocity of the ion of the same sign as the colloid may be of some importance in determining its action in holding the colloid in solution, so that of two ions of the same solution tension that moving faster is the more powerful. Whether the dissolving action of the hydrate, which is much greater than is calculated from its tension coefficient, is due to its great velocity, or, as appears perhaps more probable, to the presence in the hydrate of oxygen ions of low solution tension, cannot be definitely stated. It is possible both factors are to be considered.

VI. Quantitative Relationships.

It would seem that a certain amount of work must be done to precipitate a given amount of colloid. Since the tension coefficient is the potential factor of the energy and the concentration of the ions the quantitative factor, I think the relationship should hold, for precipitating salts having ions of the same valence, that the product of

the concentration and the tension coefficient should be a constant; or, since the tension coefficient of the colloid itself is not in this case zero, the formula should be

$$C \times (\pi_{salt} - \pi_{colloid}) = K.$$

At first glance at Table II, this relationship does not appear to hold. Lead chloride and particularly zinc chloride are far more powerful than the formula requires. Zinc chloride precipitates more powerfully than cobalt chloride, whereas the tension coefficient of the cobalt is greater than that of the zinc. The salts exhibit exactly these same exceptions in their toxic action toward protoplasm.[1] The explanation of these exceptions is probably the hydrolytic decomposition of the salts. If zinc chloride is added to an alkaline albumin solution, the hydrochloric acid set free by hydrolysis will be neutralized by the hydrate and new acid will be formed. While there is not a great difference (see Ley) between the degree of hydrolysis of zinc chloride and cobalt chloride, there is a great difference in the amount of acid which can be titrated in the two solutions owing to the difference in solubility of the hydrates. Zinc hydrate is so insoluble that it falls out of solution and nearly the whole of the chloride can be set free as acid. This is not the case with the cobalt. When a heavy metal salt solution is added to albumin, a considerable quantity of acid is in this way added, as well as the salt. It is necessary to determine how much acid is available in each salt solution.

Ley[2] has determined this amount for lead chloride, aluminium chloride, and some other salts. I have also determined in the same way, by titrating the salt with $\frac{n}{20}$ sodium hydrate using phenolphthalein as an indicator, how much of the chlorine could be titrated as free acid. With the cobalt salts the color of the cobalt makes the determination somewhat uncertain, but the figures given below represent in the case of cobalt chloride certainly a maximum. The following results were obtained:

	Per cent of chlorine titrated as acid.		Per cent of chlorine titrated as acid.
$PbCl_2$ . . .	80.0	$NiCl_2$ . . .	90.0
$CuCl_2$ . . .	95.0	$MnCl_2$ . . .	00.9
$AlCl_3$ . . .	98.0	$ZnCl_2$ . . .	99.0
$CdCl_2$ . . .	65.0	$HgCl_2$ . . .	86.0
$CoCl_2$ . . .	7.0		

[1] MATHEWS: This journal, 1904, x, p. 298.

[2] LEY: Zeitschrift für physikalische Chemie, 1899, xxx, p. 206.

A part of the precipitating energy of the salt solution is thus derived from the acid formed by hydrolysis.

Besides adding to the energy of the salt that of this acid, a correction must be made for the incomplete dissociation of the salt, since the ionic concentration, and not the molecular, is required. With these corrections, the results were obtained which are given in Table VII.

TABLE VII.

$C \times (\pi_{SALT} - \pi_{COLLOID}) = K.$

Salt.	C Salt.	C_1 cation ionic.	C_2 HCl.	π_1 Salt.	π_2 HCl.	$\times \frac{E}{(E_{salt\ cat.})}$	$\times \frac{E}{(E_{acid\ cat.})}$	K
HCl			0.001		1.287		0.00138	0.00138
$MgCl_2$	0.03 *n*	0.01200	0	+0.165 −0.136 +0.014	..	0.00273	..	0.00273
$MnCl_2$	0.0058 *n*	0.00239	0	0.354	..	0.00237	..	0.00237
$NiCl_2$	0.00136 *n*	0.00058	0.00100	0.956	1.287	0.00110	0.00128	0.00238
$CoCl_2$	0.00258	0.00106	0.00013	0.950	1.287	0.00212	0.00017	0.00239
$CdCl_2$	0.00066	0.00033	0.00043	0.773	1.287	0.00067	0.00059	0.00126(?)
$PbCl_2$	0.00060	0.00030	0.00048	1.033	1.287	0.00068	0.00066	0.00134
							Mean value of K =	0.00208

The results show a constant value for magnesium, manganese, nickel, and cobalt, but lead and cadmium are more powerful than they should be. The explanation of the exceptional position of these elements is not clear. They showed, also, an exceptional toxicity toward protoplasm.

If K of any colloid is known and the concentration of salt necessary to precipitate it is determined, π of that salt may be calculated. If the foregoing reasoning and conclusions concerning colloidal precipitation are correct, an additional means is thus given for the determination of the solution tension of an ion. If, for example, calcium chloride be calculated in this way, a value of 1.799 volts is obtained for its solution tension in normal solution. This number is considerably lower than that calculated from the heat of formation of the salt

which is given by Wilsmore as 2.3 volts. As the calculation from the heat of formation is uncertain, magnesium, for example, if calculated in this way giving a value also of 2.3, it is not impossible that 1.799 volts is nearer the true value of the solution tension of this ion.

The determination of the solution tension of the colloid may possibly be made, when $\pi_{colloid}$ has been determined, by the formula of the tension coefficient of the colloid. π of boiled egg-albumin is in solutions of the alkalinity used about —0.1 volt, since salts of a higher tension coefficient precipitate and those of a lower dissolve this colloid. If the concentration and solution tension of the cation attached to the colloid is known, that of the colloid may be found.

In conclusion, there is one other factor to be mentioned which may play a part in colloidal precipitation. It has been observed by Freundlich that there is a time element in the precipitation of colloids. That is, if a considerable quantity of salt is added at once, less is required to precipitate than if small quantities are added at successive intervals. It seems that concentration may play a part in the precipitation. I have frequently observed that the first effect of adding strong solutions may be, in the case of some salts, a solution of the colloid; while precipitation comes slowly after a time, or at once if weak solutions are used. This is a matter I have not particularly investigated, although it seems to me not unlikely that it may play an important part in the action of salts on protoplasm. We may be dealing here with a concentration chain effect, and it may be possible to apply to this case Planck's formula for the difference in potential between two electrolyte solutions where the electrolytes are different and the concentration the same, the colloidal ion being regarded as having no velocity at all.

I hope to carry the investigation somewhat further in the direction particularly of the antagonistic action of different salts on colloidal solutions and the resemblance of these phenomena to the phenomena of nerve stimulation.[1]

Summary.

1. The precipitation of colloidal egg-albumin, and possibly other colloids, is brought about chiefly by an alteration in the surface energy

[1] A friend and former colleague, Dr. C. H. Nielson, has informed me that he has also reached the conclusion that both solution tension and valence of the precipitating ion play a part in its action. Our work has been quite independent and is, I believe, mutually confirmatory on these points.

of the colloid. The precipitating power of an electrolyte is determined primarily by the solution tensions of the ions. The ion of opposite sign to the colloid precipitates; the ion of the same sign dissolves the colloid, and is not inert, as is often stated. This confirms Pauli's results and my own conclusions upon the physiological action of the ions. The precipitating power of the ion of opposite sign and the dissolving power of the ion of the same sign is in each case inversely proportional to its solution tension.

2. The action of any electrolyte, whether dissolving or precipitating upon colloidal albumin, is determined by the fact that the anion or the cation predominates in its action. The oxidizing or reducing powers of any salt, and their precipitating or dissolving powers, and their toxic action on protoplasm are determined by the formula:

$$\pi = \frac{\epsilon_a - \epsilon_c}{\epsilon_a + \epsilon_c}.$$

In this formula ϵ_a and ϵ_c are the solution tensions respectively of anion and cation in the given concentration. The factor π is called the *tension coefficient* of the salt.

3. If π of the salt is greater, that is, more positive, than π of the colloid, when the colloid is electro-negative, the salt will precipitate the colloid; if it is less than π_{colloid}, the salt dissolves the colloid.

4. The difference in precipitating power of salts having ions of the same valence is measured by the difference between their tension coefficients and that of the colloid.

5. The valence of the ion of the same sign as the colloid is without importance in determining the action of this ion; the valence of the ion of the opposite sign to the colloid is of importance. If the valence is greater than unity, the effect of the polyvalence is to increase the precipitating power of the salt. The ion acts as if its solution tension was lower than it is. It is possible that a correction can be made to the foregoing formula by substituting $\epsilon_c - A\ (n-1)$ in place of ϵ_c in the numerator of the tension coefficient. In this expression ϵ_c is the solution tension of the ion of opposite sign to the colloid; A is some positive number; and n the valence of the ion. The results of this investigation do not permit a calculation of A.

6. As the valence of the ion of the same sign as the colloid is without importance, and as this ion has an opposite action to the precipitating ion, it appears that Whetham's explanation of the importance of valence is incorrect. It is suggested that the valence of the

polyvalent precipitating ion is of importance because such an ion is able to combine with several colloidal particles forming larger aggregations than an univalent salt of the same tension coefficient. Valence influences flocking, according to this explanation, by influencing the mass and not the surface energy of the colloidal particles.

7. The hydrolytic dissociation of the salts of the heavy metals is an important factor in their action.

8. For salts having precipitating ions of the same valence, the concentration of the ions of the salt necessary to precipitate a given amount of colloid multiplied by the tension coefficient may be expected to give a constant. This was found to be approximately true.

THE CHEMISTRY OF MALIGNANT GROWTHS. IV. — THE PENTOSE CONTENT OF TUMORS.

By S. P. BEEBE and PHILIP SHAFFER.

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SINCE the rather well-known observation of Hammarsten[1] that a reducing pentose is one of the cleavage products of pancreas nucleoproteid, considerable attention has been paid to the determination of the amount of pentose in various nucleoproteids as well as in various animal tissues.[2] Blumenthal[3] in 1897 extended the observation of Hammarsten by finding pentose among the decomposition products of the nucleoproteids of the thymus, thyroid, brain, spleen, liver, etc. Grund[4] made a number of determinations of pentose in dried animal tissues and calculated the amount of pentose in each organ. Bendix and Ebstein[5] published a little later a similar investigation in which they obtained nearly the same results. In the determination of the pentose in human organs obtained at autopsy these authors obtained lower results than those for freshly examined animal organs, and in a later paper Bendix[6] reported experiments showing that putrefaction destroys the pentose of the tissues. On this account he expressed the opinion that determinations of pentose in organs, especially the liver, obtained at autopsy from forty-eight to ninety-six hours after death, which is not uncommon, are of little value.

[1] Hammarsten: Zeitschrift für physiologische Chemie, 1894, xix, p. 28.

[2] For a résumé of the literature regarding pentose in the animal organism see Wohlgemuth: Biochemisches Centralblatt, 1903, i, p. 533; also Neuberg: Ergebnisse der Physiologie, 1904, iii, p. 373.

[3] Blumenthal: Zeitschrift für klinische Medizin, 1898, xxxiv, p. 166.

[4] Grund: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 111.

[5] Bendix and Ebstein: Zeitschrift für allgemeine Physiologie, 1902, ii, p. 2.

[6] Bendix: Zeitschrift für dietätische und physikalische Therapie, 1903, iii, p. 26.

It should be noted that these authors claim the destruction of pentose is caused by putrefaction, *i. e.*, by bacterial contamination. There is no evidence to show that such destruction takes place on pure autolysis. Many of the growths from which our analyses were made were received at the laboratory fresh from operation, but in all tumors which have attained any size one finds products of autolysis. Such a condition is very probably not accompanied by the destruction of pentose noted by Bendix and Ebstein.

A number of investigators have caused it to be believed that the pentose which is found among the cleavage products of animal tissues is derived from the nucleoproteids. Although it is still an unsettled question whether nucleoproteids do not exist also in the protoplasm of the cell and in extracellular localities, it seems fairly certain that they are largely characteristic of the cell nucleus, functionally the most important part of the cell. In making a chemical study of tumor tissues it is important to determine whether the nuclear material contained therein is in any way different from that contained in normal cells, for unquestionably the nucleus is largely concerned in the peculiar metabolism of malignant growths. It seemed therefore of interest to determine the amount of pentose in a number of tumors. In some cases the apparently normal tissue surrounding the growth was analyzed for comparison. The total phosphorus which is likewise chiefly found in the nucleoproteids was also determined.

Attention has been called recently to the pentose in tumors by Neuberg, who claims to have found a reducing pentose[1] by the autolysis of a carcinoma of the liver, while he was not able to find such a product from the autolysis of normal liver tissue. Further, he has found that the secondary growth in the liver yielded pentose on autolysis, while the primary growth in the stomach did not.[2] He has made only one observation in each case, so that it is impossible to say how true this principle will hold. His work must be accepted rather as a suggestion than as a demonstrated fact.

The Tollens[3] method was used for the determination of the pentose and was carried out briefly as follows. The tissue, previously dried at 100° and extracted thoroughly with ether, was distilled with 150 cubic centimetres of 12 per cent hydrochloric acid until 75 cubic centimetres of distillate had been obtained. 75 cubic centimetres of

[1] NEUBERG: Berliner klinische Wochenschrift, 1904, xl, p. 1080.
[2] NEUBERG: Berliner klinische Wochenschrift, 1905, v, p. 118.
[3] TOLLENS: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 239.

12 per cent hydrochloric acid were added, and another 75 cubic centimetres distilled over. This process was repeated until 400–450 cubic centimetres of the distillate were obtained. The distillate was filtered from the fatty acids which were invariably present in considerable quantities even when the tissue had been thoroughly extracted with ether, the filter washed with about 50 cubic centimetres 12 per cent hydrochloric acid, and the furfurol precipitated in the filtrate as the phloroglucid by the addition of a 0.5 per cent solution of phloroglucin in 12 per cent hydrochloric acid. After standing from forty-eight to ninety-six hours, the precipitate was filtered on dried weighed filters (S and S, 589, white ribbon), washed free from acid, dried at 100° and weighed. From the weight of the phloroglucid the amount of pentose was calculated by the arbitrary method of Tollens. The results were calculated as L-xylose, which is according to Salkowski and Neuberg[1] the pentose derived from the nucleoproteids of the pancreas. According to Wohlgemuth[2] L-xylose is the pentose derived from the liver nucleoproteid, and so far no other pentose has been identified as being derived from the nucleoproteids of animal origin.

A correction which we have added to the weight of the phloroglucid in our calculations needs a brief explanation. The difficulty of obtaining accurate results in weighing precipitates on dried filters is notorious. The filters which we used (see above), were dried at 105° in weighing bottles and weighed after cooling in a desiccator — both before and after filtering. It was found that such filters invariably lost in weight by washing with 500 cubic centimetres 12 per cent hydrochloric acid, and then with water till free from acid. In seven filters treated in this way the loss varied between 0.0008 and 0.0030 gm., the average loss being 0.0019. This amount has therefore been added to the weight of the phloroglucid in each case. By the method of calculating the pentose proposed by Tollens, a correction of 0.0052 gm. is added to the weight of the phloroglucid, and the sum is multiplied by 0.92, which gives the weight of the xylose. We have added (0.0052 + 0.0019 =) 0.0071 gm. to the weight of the phloroglucid and multiplied the sum by 0.92. This method of calculation is of course not based upon any theoretical grounds, but upon factors which have been determined by experiment. It is probable that the results do not represent great accuracy, but

[1] SALKOWSKI and NEUBERG: Berichte der deutschen chemischen Gesellschaft, 1902, xxxv, p. 1467.

[2] WOHLGEMUTH: Zeitschrift für physiologische Chemie, 1902, xxxvii, p. 475.

for comparative purposes it is believed that they are sufficiently reliable. The differences between many of our results are marked and well beyond the limits of error in the methods.

The phosphorus was determined by the Neumann[1] method upon the same tissues as were used for determining the pentose. Both phosphorus and pentose were calculated as percentages of the dried, fat free tissues.

The results show no striking differences from what may be called the normal content of pentose. In general the amount of pentose

Tissue.	Per cent xylose.	Per cent phosphorus.	Ratio of xylose-phosphorus.
1. Normal breast	0.23	0.27	85
2. Normal breast (two months pregnant)	0.29	0.22	132
3. Normal secreting breast (case of puerperal septicemia)	0.27	0.42	64
4. Normal breast tissue (from a number of carcinoma cases)	0.22	0.27	81
5. Carcinoma of the breast	0.41	0.59	70
6. Scirrhus carcinoma of the breast	0.44	0.74	59
7. Scirrhus carcinoma of the breast. (The tissue in this case had been extracted with salt solution before drying)	0.66	0.59	112

was larger than is usually found in normal tissues of the corresponding organ. This might be explained on the ground of there being more nuclear material in the tumor tissue, but this explanation does not apply to all cases.

In these breast cases it is seen that the normal content of pentose in the resting gland is about 0.23 per cent, and this figure is slightly increased during secretion. In one case the normal gland tissue surrounding the carcinoma contained so small a quantity of pentose that no phloroglucid precipitate could be obtained in the hydrochloric acid distillate. In the carcinoma tissue the pentose is nearly doubled and in the last scirrhus growth nearly trebled. The curious part of these findings is that the scirrhus tissue contained a great deal more pentose than was found in the simple carcinoma, although morphologically the latter has much more nuclear material. We

[1] NEUMANN: Zeitschrift für physiologische Chemie, 1902, xxxvii, p. 115.

must either suppose, then, that the nucleic acids of the growths are variously constituted, or that some pentose in the case of the scirrhus growths has been derived in part from the extra-nuclear portions of the tissue. The last tumor was a very large growth of its kind, but the total quantity of nuclear material was small. It seems hardly probable that we can establish any definite figure as the usual pentose content for a breast carcinoma. There is a considerable variation in the pentose content of normal organs, and it is probable that this holds true for malignant growths.

Tissue.	Per cent xylose.	Per cent phosphorus.	Ratio of xylose-phosphorus.
1. Normal liver No. 1	0.38	1.04	36.0
2. Normal liver No. 2	0.39	1.065	36.0
3. Carcinoma of the liver. (Primary in the liver)	0.33	0.93	35.0
4. Carcinoma of the liver. (Primary in the breast)	0.21	1.08	19.5
5. Carcinoma of the liver. (Primary in the stomach)	0.42	1.18	36.0
6. Carcinoma of the stomach. (Not connected with the above case)	0.46	1.20	38.0
7. Carcinoma of the liver. (Primary in the pancreas)	0.68	0.94	72.0
8. Normal liver tissue in the above case .	0.63	1.03	61.0

There is no organ in which metastases are more likely to form than in the liver, and we have analyzed a number of growths from this organ in the hope of finding some relationship between the primary and the secondary growths. It is rarely possible to get good samples of both tissues from the same patient for analysis, so that we have had to compare the primary growth in one patient with a similar secondary growth in some other patient.

Very little generalization can be made from these cases because of the great differences which they show. Some interesting comparisons may be made, however. Attention is called to the first three analyses shown in the preceding table, where it is seen that the figures obtained for normal liver tissue are very close to those obtained in the growth which was primary in the liver. The normal liver tissues cited above came from healthy persons of thirty-four and forty years who had been accidentally killed. They differ, indeed,

from the figures obtained by Grund,[1] who found the liver to contain 0.56 per cent pentose reckoned as xylose, but our analyses were in all cases made on human tissues which had been obtained in good condition. It is hardly probable that liver tissue from calves is in chemical constitution precisely like the human organ. This carcinoma, primary in the liver, was a case in which nearly all of the liver tissue had been replaced by carcinoma, so little pure normal tissue being left that we could not get a sufficient amount for these analyses. It is difficult to understand how life could have existed under such circumstances long enough to permit the development of so extensive a growth, unless one assumes that the tumor cells performed some of the functions of normal hepatic cells. This is the only tumor primary in the liver that we have been able to obtain. The close similarity between the tumor tissue and the normal tissue in both pentose and phosphorus in this liver case stands in striking contrast to the findings in the breast cases.

In contrast to all the others in this group is number 4, a growth primary in the breast, which shows a low pentose content very similar to normal breast tissue. (See first table.) The phosphorus content is that of normal liver tissue, while the pentose is very nearly identical with normal tissue surrounding the primary growth.

In number 5, the carcinoma of the liver which was primary in the stomach, we found a pentose and phosphorus content which is almost identical with that found by the analysis of a primary carcinoma of the stomach. The figures in this case and the third of the above tables lead to the suggestion that the pentose content of the metastases is very similar to that of the primary growth. Such a finding would be well in accord with the usual explanation of the formation of metastases. Indeed, if one accepts the explanation for the formation of metastases by the transferring of cells from the primary growth through the various paths of the circulation, one is almost forced to the belief that the secondary growths should have an identical composition with the primary tissue. One of us has, however, recently expressed the opinion that the secondary growth may be influenced somewhat by the tissues which surround it. The presence of nucleohiston in the lymph gland metastases found by Bang and also by one of us[2] was explained in this way.

It is possible that the figures shown in the last two analyses of the table given above may be reasonably explained by such a prin-

[1] GRUND: *Loc. cit.* [2] BEEBE: This journal, 1905, xiii, p. 341.

ciple. The tumor, primary in the tail of the pancreas, does not show the high pentose content one would expect from a growth in this organ, but it does resemble very closely the normal liver tissue surrounding the nodules in the liver. This normal tissue is not to be understood as normal in the same sense that numbers 1 and 2 of the above table are. Unfortunately we could get none of the primary growth for analysis, so that our opinion regarding it is based upon what we suppose would be its pentose content from analogy with the high percentage obtained from normal pancreas tissue. After all, it is a mere conjecture. One thing does appear plain from these analyses. The phosphorus is quite constant, while the pentose shows wide variation, and it is quite impossible to establish any figure as being normal for carcinoma of the liver. Probably further analyses will show a pretty definite relation between the content of pentose in the primary growth and the corresponding secondary growths in the liver.

In order to determine whether such a process as fatty degeneration would have a marked influence on the pentose content, two livers from cases of acute yellow atrophy were subjected to the same processes of analyses as the tumors.

Acute yellow atrophy.	Per cent xylose.	Per cent phosphorus.	Ratio of xylose-phosphorus.
1. But little degenerated . .	0.51	0.96	53
2. Badly degenerated . . .	0.46	1.26	36

The percentage of pentose is higher than was found for perfectly normal liver tissue. It is possible that in these livers some of the proteid has been changed to an ether soluble substance which would be removed in the course of preparing the tissue for analysis, and the pentose, although unchanged in absolute amount, would form a higher percentage of the tissue which remained after extraction with ether. The fact that we obtained a lower percentage of pentose in the liver which contained the more fat by 20 per cent speaks decidedly against such an explanation, and we are of the opinion that such a process of autolytic degeneration as we have in these livers probably has little effect on the percentage of pentose.

A number of other tumors which do not form a related series were

analyzed. The difficulty of getting suitable material for comparison and corroboration has led us to include the results in this paper.

Tissue.	Per cent xylose.	Per cent phosphorus.	Ratio of xylose-phosphorus.
Carcinoma of the ovary . .	0.78	1.00	78
Melanoma	0.55	0.98	56
Hypertrophied testicle . . .	0.43	0.48	90
Epithelioma	0.25	0.83	30
Round cell sarcoma	0.66	....	..

The tumor in this list giving the highest pentose percentage was badly degenerated. In no case has there been evidence that a degenerated condition of the tissue is accompanied by low pentose figures.

The danger of drawing conclusions from few analyses is well illustrated by the figures given herewith. If we had been unfortunate enough to have analyzed only the first three tissues given in the second table, an interesting theory might have seemed to have convincing evidence. The great need in the study of malignant growths is additional facts; there are plenty of theories. Our analyses are communicated rather because they represent additional facts of a nature not easy to get than because they throw any striking light on the present discussions of the cancer problem.

ON THE UTILIZATION OF VARIOUS CARBOHYDRATES WITHOUT INTERVENTION OF THE ALIMENTARY DIGESTIVE PROCESSES.

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IT is commonly accepted that carbohydrates must be converted into monosaccharides before they can enter to any extent into the processes of intermediary metabolism. This assumption is based in general upon two series of experimental observations, namely, that (1) only those poly- and disaccharides which can undergo such cleavage in the body serve as glycogen formers; and (2) when these carbohydrates are introduced into the circulation without previous digestive changes they may reappear in large proportions in the urine, unless the blood contains appropriate enzymes to convert them into dextrose and similar sugars. The monosaccharides are thus primarily the "physiological" carbohydrates capable of direct utilization in the organism of higher animals.

The assimilation of carbohydrates introduced into the body in a *parenteral* way, that is, with avoidance of the alimentary digestive tract, has been studied by several investigators. Böhm and Hoffmann [1] injected glycogen in doses of 3 to 10 gm. slowly into the circulation of cats. The urine subsequently contained, in addition to a reducing compound (presumably dextrose), a substance apparently identical with achroodextrin and producing a dextrorotation of +194°. The solutions of the urine carbohydrate were not opalescent and gave no color with iodine solution. Dastre and Bourquelot [2] studied the utilization of maltose introduced subcutaneously and intravenously in animals; they found it to compare favorably with that of dextrose and to be far better than that of cane sugar. Maltose was recovered in the urine in quantities ranging from 8 to 30 per

[1] Böhm and Hoffmann: Archiv für experimentelle Pathologie und Pharmakologie, 1877, vii, p. 489.

[2] Dastre and Bourquelot: Comptes rendus, 1884, xcviii, p. 1604.

cent; the amounts of saccharose recovered varied from 30 to 95 per cent. Dastre[1] subsequently examined the direct utilization (or retention) of lactose and its inversion products, galactose and dextrose, by the methods of intravenous injection and perfusion of organs. The lactose was scarcely retained, and Dastre groups the sugars investigated in the following series in the order of increasing utilization: saccharose, lactose—maltose—galactose, dextrose. In his classic paper on glycogen formation, C. Voit[2] has recorded experiments (by G. Lusk) on the relative amounts of glycogen found in the liver after subcutaneous administration of various sugars to rabbits in 50 gm. portions, as follows:

After dextrose injections the liver contained				5.0	per cent.
"	levulose	"	"	5.9	"
"	saccharose	"	"	0.7	"
"	lactose	"	"	0.8	"

The loss of sugars in the urine was least in the case of dextrose and greatest with lactose. Subsequently F. Voit[3] made a careful study of the excretion of various sugars by the kidneys after subcutaneous administration in man. The doses approximated 10 gm., although they ranged from 1.5 to 25 gm. for some of the carbohydrates. In the case of dextrose, fructose, galactose, and maltose practically no sugar was recovered; saccharose and lactose were eliminated almost quantitatively. The pentoses were poorly utilized, and reappeared in about one-half the original quantity. Of sorbinose, which is not attacked by yeast, 36 per cent was recovered; while of the raffinose injected two-thirds or more reappeared in changed form in the urine. In connection with our experiments, particular interest is attached to F. Voit's experience with polysaccharides. The quantities eliminated in the urine are tabulated below:

Glycogen (one trial)	none.
Erythrodextrin (one trial)	14 per cent.
Achroodextrin (two trials)	24 and 34 per cent.
Amylodextrin (three trials)	10, 15, and 28 per cent.

[1] DASTRE: Archives de physiologie, 1889, p. 718; 1890, p. 103.

[2] VOIT, C.: Zeitschrift für Biologie, 1892, xxviii, p. 245.

[3] VOIT, F.: Münchener medicinische Wochenschrift, 1896, p. 717; Deutsches Archiv für klinische Medicin, 1897, lviii, p. 521.

Teissier and Zaky[1] administered repeated small doses of glycogen intravenously to rabbits until a total of 1 to 1.5 gm. per kilo was introduced. Glycogen was not observed in the urine, although sugar was once reported. The animals died.

Various investigators have considered the absolute quantities of different sugars which can be introduced into the body per os or parenterally without loss of carbohydrate in the urine. The most recent study, by Blumenthal,[2] has established the limits for the complete assimilation of a number of familiar sugars after intravenous infusions in rabbits as follows:

For dextrose	2.0–2.8	grams.
" fructose	2.4–2.7	"
" galactose	0.4–0.6	"
" saccharose	0.3	"
" lactose	0.25	"

This relatively poor utilization of the disaccharides corresponds with the experience of other investigators as already noted, and plainly indicates the importance of inversion of these sugars prior to their metabolism.

Our attention has been directed to this subject in connection with the recent discussions on subcutaneous nutrition.[3] P. Mayer[4] reports v. Leube and Gürber as having found in animal experiments that dextrin, administered subcutaneously, was readily absorbed, but reappeared unconverted in the urine; 15 per cent solutions of glycogen, on the other hand, were assimilated under similar conditions without elimination of any appreciable carbohydrate in the urine. Mayer has repeated these experiments on rabbits with erythrodextrin and glycogen. He found that 34 to 50 per cent of the subcutaneously introduced dextrin escaped oxidation and could be recovered from the urine in the changed form of achroodextrin, although twice the quantity used (10 gm.) could be administered per os without yielding a trace of carbohydrate in the urine. Glycogen was

[1] TEISSIER and ZAKY: Comptes rendus de la société de biologie, 1902, liv, p. 1098.

[2] BLUMENTHAL: Beiträge zur chemischen Physiologie, 1905, vi, p. 329. The literature is reviewed in this paper.

[3] Cf. v. LEUBE: von Leyden's Handbuch der Ernährungstherapie, 1903, i, p. 393; MARIANI: Jahresbericht für Thierchemie, 1897, xxvii, p. 577; CORRADI: *ibid.*, 1898, xxviii, p. 513; GUMPRECHT: *ibid.*, 1898, xxviii, p. 514.

[4] MAYER: Fortschritte der Medicin, 1903, xxi, p. 417.

given subcutaneously in quantities of 5 gm. without any loss through the kidneys.

The difference here reported between the physiological behavior of glycogen and dextrin, in correspondence with F. Voit's experience on man, is noteworthy. The theoretical consequences have already been pointed out by Mayer. Either the glycogen is used directly in the organism without conversion into dextrose, or the dextrin formed from glycogen in hydrolysis by the animal enzymes differs from the ordinary dextrin obtained by digestion of starch in the laboratory. It appears as if dextrins are not utilized without preliminary conversion to sugar, and the blood contains no specific dextrinase. An amylase is known to occur in the blood; "and since, as far as we are aware, starch cannot be converted to sugar except through the stage of dextrins, contradictions arise which future investigation alone can explain."

A repetition of the injection experiments with glycogen has given us somewhat different results indicating no such profound difference between this carbohydrate and dextrin in its parenteral utilization. The trials were conducted on rabbits, cats, and dogs, both subcutaneous and intraperitoneal injections being employed. In view of the results noted, the experiments were extended to include a number of other polysaccharides: soluble starch (amidulin), inulin, isolichenin, as well as ovomucoid and saccharose. The carbohydrates were injected in sterile solutions. The urine, removed by catheter from the dog and collected from the other animals in cages, was always examined in the polariscope, after decolorization with lead acetate; reduction tests were also made upon the fresh urine, and the carbohydrates were isolated wherever the quantities permitted. The urine was collected in every case until no appreciable rotation was obtained. The rate of elimination varied considerably with the different carbohydrates. Only a few of the protocols are reproduced here in detail.

Injection of glycogen. Subcutaneous. *Experiment 10.* — A medium-sized rabbit received 2.1 gm. of glycogen in solution. The urine of the following day (80 c.c.) gave a rotation of $+0.55^{\circ}$ in a 2 dcm. tube. Calculating the rotation as due to glycogen ($[\alpha]_D = +196^{\circ}$ according to Huppert) 0.11 gm. was recovered. The urine gave no direct reduction with Fehling's solution. The exact nature of the excreted carbohydrate was not determined. The urine subsequently showed no rotation.

Experiment 3. — A rabbit weighing 2.7 kilos received 2.4 gm. of glycogen dissolved in 30 c.c. of water. Calculated as glycogen from the dextro-

rotation of the urine, 0.29 gm. was recovered in the two days following. The urine gave no reduction test.

Experiments 1 and 12. — A rabbit weighing 2.7 kilos received 30 c.c. of an 8 per cent glycogen solution. On the day following the urine collected gave no rotation. The urine of the second day (200 c.c.) gave a rotation in a 2 dcm. tube = +0.66°, equivalent to 0.3 gm. glycogen. No sugar was present. Another small rabbit, observed one day only, eliminated 100 c.c. urine with a rotation of +0.4° (2 dcm. tube) = 0.1 gm. glycogen, after receiving 2.1 gm. The animal died before the experiment was completed.

Experiment 7. — A small cat received 1.74 gm. of glycogen dissolved in 30 c.c. of water. The observations were discontinued before the urine was entirely devoid of rotating power. Within two days, however, the urine showed rotation equivalent to 0.32 gm. of glycogen.

Intraperitoneal. *Experiment 24.* — A bitch weighing 8½ kilos received 2.8 gm. of glycogen in solution. The urine of the next thirteen hours gave a rotation equivalent to 0.09 gm. of glycogen. Subsequently the urine was normal.

Experiments 11 and 13. — A small rabbit received an injection of 2.16 gm. of glycogen, and a rotation equivalent to 0.26 gm. glycogen was observed within the following two days. When 4.6 gm. of glycogen were injected into another small rabbit (1.5 kilos in weight) the equivalent of 0.54 gm. was similarly recovered within forty-eight hours.

Experiment 14. — The rabbit used in Experiment 13 received 4 gm. of glycogen. The urine indicated an excretion of 0.68 gm. glycogen.

The urines from Experiments 13 and 14 were united, treated with lead acetate and filtered. After removal of the excess of lead from the filtrate with hydrogen sulphide, the fluid was concentrated and precipitated with alcohol. The precipitate, dissolved in water, gave no characteristic color with iodine solution and did not reduce Fehling's solution until it had been inverted with acid. The transformation product of glycogen therefore resembles achroodextrin in correspondence with observations of Böhm and Hoffmann. In the preceding protocols the output of carbohydrate has been calculated by the use of the factor for the specific rotation of glycogen ($[\alpha]_D = +196°$). In view of the lower specific rotation of dextrins (+180°) the figures for excreted carbohydrate are, if anything, too small.

Injection of dextrins. Subcutaneous. — The dextrin used was prepared by digestion of starch with saliva, being precipitated from the products of amylolysis by alcohol and purified by repeated precipitation. Dissolved in urine it afforded a dextro-rotation over three times as great as that of dextrose. Dextrin was recovered as such from the united urines of Experiments 2 and 3 below, by the method just described under glycogen.

Experiments 2 and 3. — A rabbit weighing 2700 gm. received 2.5 gm. (in 24 c.c.) and 2.0 gm. (in 24 c.c.) respectively in two trials. The corresponding urines contained 0.59 gm. and 0.22 gm. of dextrin, and gave negative reduction and fermentation tests.

Experiments 4 and 6. — A small cat received 3.5 gm. (in 30 c.c.) and 2.0 gm. (in 20 c.c.) in two separate trials. The corresponding urines were free from fermentable or reducing sugar and showed rotations equivalent to 0.88 gm. and 0.45 gm. of dextrin respectively. By benzoylation of the combined urines and subsequent saponification of the benzoate with sodium ethylate, a small quantity of dextrin-like substance was isolated.[1]

Injection of soluble starch. Intraperitoneal. — Soluble starch (amidulin) was prepared according to the method of Syniewski[2] and after hydrolysis yielded dextrose equivalent to 84.4 per cent of starch. The specific rotation of the carbohydrate was determined ($[a]_D = +199°$) and the output of soluble starch in the urine estimated on the basis of the data thus obtained.

Experiment 18. — A small rabbit received 2.5 gm. of the soluble starch. The urine of the following day (130 c.c.) gave a rotation of $+2.13°$ in a 2 dcm. tube. An equivalent of 0.76 gm. soluble starch was recovered.

Experiment 19. — A bitch weighing 8½ kilos received 75 c.c. of a solution containing 6.4 gm. of soluble starch. A total of 0.43 gm. was recovered within fifteen hours.

From the urine of Experiment 18 a dextrin-like substance was isolated. It gave a purple-blue reaction with iodine solution, no reduction previous to hydrolysis, and was sufficiently pure to indicate a specific rotation of $+174.6°$. Considering the difficulty of any sat-

[1] Cf. LEMAIRE: Zeitschrift für physiologische Chemie, 1896, xxi, p. 442 for methods.

[2] SYNIEWSKI: Berichte der deutschen chemischen Gesellschaft, 1897, xxx, p. 2415.

isfactory isolation from urine, it is probable that the product excreted represents the injected carbohydrate altered only slightly, if at all.

Injection of inulin. Intraperitoneal. — A study of the behavior of inulin in this connection is of especial interest, since no inulase has yet been found in the animal body,[1] although the carbohydrate is readily hydrolyzed to levulose by means of dilute acids. The glycogen-forming properties of inulin are likewise uncertain or minimal.[2] Warm solutions were injected into rabbits. In the estimation of the output of inulin the specific rotation ($[a]_D = -39.5°$) corresponding with the results of Dean's observations was applied.[3]

Experiments 15 and 16. — A small rabbit received 2.8 gm. of inulin in 40 c.c. of water. The urine of the next thirty-six hours contained 2.2 gm. of inulin. It gave no reduction with Fehling's solution. The same animal subsequently received 2.2 gm. of inulin. The urine of the next twenty-four hours (350 c.c.) gave no appreciable reduction; but after being cooled, it deposited characteristic spheroids of inulin. The urine was hydrolyzed with one-half per cent sulphuric acid — one and one-half hours on a water-bath — and after neutralization the inulin was estimated from the levulose determined by Allihn's gravimetric copper method. The inulin recovered was 1.43 gm.

Injection of isolichenin. Intraperitoneal. — The preparation and properties of this carbohydrate from Iceland moss (Cetraria islandica), resembling soluble starch in some respects, have been described in a previous paper from this laboratory.[4] Dextrose is formed from it by hydrolysis with acids; isolichenin, however, is peculiar in its resistance towards ordinary amylolytic enzymes.[5] The carbohydrate in the urine was roughly calculated as dextrin ($[a]_D = +180°$) from the observed rotation.

Experiment 17. — After injection of 1.76 gm. of isolichenin in a rabbit the urine of the next twenty-four hours (90 c.c.) gave a rotation + 0.8° in a 2 dcm. tube = 0.2 gm. as dextrin. The experiment was here interrupted. Subsequent experience showed that the elimination of this carbohydrate is very slow, hence the entire output was probably not obtained.

[1] Cf. CHITTENDEN: This journal, 1898, ii, p. xvii; RICHAUD: Comptes rendus de la société de biologie, 1900, lii, p. 416; BIERI and PORTIER: *ibid.*, p. 423. For the properties of inulase cf. DEAN: Botanical Gazette, 1903, xxxv, p. 24.

[2] MENDEL and NAKASEKO: This journal, 1900, iv. p. 246.

[3] DEAN: American chemical journal, 1904, xxxii, p. 69.

[4] BROWN: This journal, 1898, i, p. 458.

[5] Most of the material used in these experiments was prepared by Mr. R. B. GIBSON.

Experiments 20, 21, and 22. — A bitch of 8½ kilos in weight received 1.5 gm. of isolichenin in 60 c.c. of water. The carbohydrate recovered was equivalent to 0.64 gm. dextrin. In a second trial with 1.15 gm. a considerable output was noted. The loss of a portion of the urine vitiated the quantitative estimation. In a third experiment with an injection of 0.8 gm., an equivalent of 0.17 gm. (as dextrin) was recovered. A dextrin-like carbohydrate was isolated from the combined urines.

Injection of saccharose. Intraperitoneal. *Experiment 23.* — For purposes of comparison the bitch weighing 8½ kilos received 3 gm. of pure saccharose. The urine which gave no reduction with Fehling's solution before heating with acid contained 1.5 gm. of saccharose within the next twenty-four hours and none thereafter.

Injection of ovomucoid. Intraperitoneal. — The glycoproteid was prepared in the usual way from hens' eggs and injected into rabbits. No reducing substance was found in the urine, although the mucoid yields nearly 30 per cent of glycosamine after hydrolysis with acids.[1]

Experiments 8 and 9. — The quantities injected were 1.4 gm. and 2.6 gm. The urines were slightly levorotatory and gave proteid reactions.

The results of these experiments are summarized in tabular form on the next page. The figures indicating the quantities of carbohydrate eliminated must be regarded at most as approximate only, in view of the various inaccuracies necessarily involved in the assumptions made and explicitly stated in the protocols. Nevertheless they may serve for purposes of comparison.

The preceding experiments have failed to indicate any profound qualitative difference between glycogen and dextrin in their parenteral[2] introduction into animals. It appears as if glycogen were retained slightly better than dextrin when all the quantitative data available are taken into account. At present we find no occasion, however, for assuming any specific peculiarity in the physiological utilization of glycogen. The failure of the organism to retain those carbohydrates which it is not fitted to transform by means of enzymes is well illustrated in the case of inulin and, less markedly, with isolichenin — both compounds not profoundly altered by ordinary amyl-

[1] COHNHEIM: Die Eiweisskörper, 1904, p. 279.

[2] We have adopted this convenient expression from OPPENHEIMER: Beiträge zur chemischen Physiologie, 1904, iv, p. 267, to indicate any mode of introducing substances into the body with avoidance of the alimentary tract; *i. e.*, by subcutaneous, intraperitoneal, or intravenous injection.

Number.	Substance injected.	Kind of injection.	Animal.	Quantity used.	Quantity recovered (calculated).	
				grams	grams	per cent
10	Glycogen	subcutaneous	rabbit	2.1	0.11	5
3	"	"	"	2.4	0.29	12
1	"	"	"	2.4	0.3	12
12	"	"	"	2.1	0.1 [1]	5 [1]
7	"	"	cat	1.74	0.32 [1]	18 [1]
24	"	intraperitoneal	bitch	2.8	0.09	3
11	"	"	rabbit	2.16	0.26	12
13	"	"	"	4.6	0.54	12
14	"	"	"	4.0	0.68	17
2	Dextrins	subcutaneous	"	2.5	0.59	23
3	"	"	"	2.0	0.22	11
4	"	"	cat	3.5	0.88	25
6	"	"	"	2.0	0.45	22
19	Soluble starch	intraperitoneal	bitch	6.4	0.43	7
18	"	"	rabbit	2.5	0.76	30
15	Inulin	"	"	2.8	2.2	78
16	"	"	"	2.2	1.43	65
17	Isolichenin	"	"	1.76	0.2 [1]	11 [1]
20	"	"	bitch	1.5	0.64	42
22	"	"	"	0.8	0.17	21
23	Saccharose	"	"	3.0	1.5	50
8	Ovomucoid	subcutaneous	rabbit	1.4	no sugar	. . .
9	"	"	"	2.6	"	. . .

[1] The experiment was incomplete. See the protocols.

olytic enzymes of animal origin. Renewed evidence is thereby afforded of the importance of the alimentary digestive changes in the metabolism of carbohydrates.

THE EFFECT OF HYPNOTICS AND ANTIPYRETICS ON THE RATE OF CATALYSIS OF HYDROGEN DIOXIDE BY KIDNEY EXTRACT.

By C. HUGH NEILSON and OLIVER P. TERRY.

[*From the Medical Department of St. Louis University.*]

THE effect of external factors, such as changes in temperature, antiseptics, anæsthetics, neutral salts, etc., has been studied by many investigators. From the numerous investigations on the effect of neutral salts on enzymatic action, it has been established that the bromides of sodium and potassium have a retarding action on the rate of enzymatic action in general, but especially on the rate of catalysis of hydrogen dioxide by tissue extracts.

The bromides have a wide field of application as therapeutic agents, among which is their use as hypnotics and nerve sedatives. As they have such a pronounced retarding action on the rate of enzymatic action, it occurred to us that it would be interesting to test the effects of hypnotics in general on the rate of catalysis of hydrogen dioxide by a watery extract of kidney.

The hypnotics used for these experiments were those commonly used as therapeutic agents to produce hypnosis. However, some of the well-known hypnotics were not used in these experiments, as they are not sufficiently soluble in water to carry out the experiments. Among these may be mentioned sulphonal, trional, tetronal, etc.

We also thought it would be interesting to test the effects of antipyretics and compare their actions with those of the hypnotics. We encountered the difficulty that, as a rule, the antipyretics are insoluble in water. Antipyrene, being the only one sufficiently soluble in water for our purpose, was therefore used.

We did not test the other nerve sedatives, such as opium, ether, chloroform, the bromides, nitrites, etc., as many of these have been worked out by other investigators. It may be said, however, that

these substances have, as a rule, a retarding action on the rate of enzymatic action.

In carrying out these experiments the method was as follows: The tissue extracts were made by grinding fresh beef kidney and extracting the pulp with water. This extract was very stable and perfectly neutral to litmus. 25 c.c. of distilled water as a control, or 25 c.c. of the solution to be tested, together with 5 c.c. of the extract, were placed in 200 c.c. wide-mouthed bottles. These bottles were fitted with a two-hole rubber stopper. A delivery tube was carried from the bottles to eudiometer tubes, and the amount of oxygen liberated was noted at the end of one and two minutes. 5 c.c. of hydrogen dioxide were put into the bottle through the second hole in the stopper, and the bottles were gently shaken during the experiment.

A control of distilled water was always made with the solution to be tested.

The results are seen in the following table:

Substance.	Concentration.											
	5%		2%		1%		½%		¼%		⅛%	
	Cubic centimetres of oxygen.											
	1 min	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.
Chloretone	..	..	..	..	..	..	7.0	12.0	7.0	13.0	9.0	14.0
Chloralamid	..	..	1.0	5.0	3.0	7.0	8.0	12.0	9.0	14.0	9.0	14.0
Chloral hydrate	0.0	1.0	4.0	7.0	8.0	12.0	9.0	13.0	9.0	14.0	9.0	14.0
Croton chloral	..	..	4.0	8.0	4.0	9.0	7.0	10.0	8.0	13.0	8.0	14.0
Bromidia	4.0	5.5	5.0	7.0	8.0	11.0	8.0	10.0	9.0	13.0	9.0	13.0
Paraldehyde	6.0	9.0	7.0	10.0	8.0	13.0	8.5	13.0	9.0	13.5	9.0	15.0
Urethane	6.0	8.0	7.0	9.0	8.5	12.5	8.0	13.0	9.0	13.5	5.5	14.5
Hedonal	..	..	..	..	7.0	12.0	8.0	13.0	9.0	14.0	9.0	14.0
Antipyrene	12.0	16.0	12.0	14.0	10.0	15.0	9.0	13.5	9.5	14.0	9.0	14.5
Water	9.0	14.0										

From these results it is seen that the general effect of the hypnotics is to retard the rate of catalysis. It will be further noticed that the degree of their retarding action is as follows. Chloretone is the most powerful. Then follows chloralamid; chloral hydrate; bromidia; croton chloral; paraldehyde; urethane; hedonal, in the order named. The strong inhibiting power of bromidia is, in all probability, due to the bromide contained in the mixture.

These hypnotics when used as therapeutic agents have approximately the following power: Chloretone is the most powerful. Then follow chloralamid and chloral hydrate, which have about the same power in producing hypnosis; then, croton chloral. The rest have about the same degree of power, with the exception of hedonal, which is a more powerful hypnotic than urethane, but its action is uncertain.

We thus see that there is a marked comparison between their actions as hypnotics and their retarding actions on the rate of catalysis of hydrogen dioxide.

It will further be seen that antipyrene has a stimulating effect on the rate of catalysis. This is interesting, but it would not be safe to assert that all antipyretics stimulate, on the basis of the action of antipyrene.

Bunge says that "life is a manifestation of the action of enzymes." It is therefore within the field of speculation that the action of hypnotics in producing hypnosis may be due to their retarding action on enzymatic activities.

It is not safe to assert that hypnotics produce their effects by retarding enzymatic action, and antipyretics their effects by stimulating enzymatic action.

The primary effect of hypnotics is that of a nerve sedative with a secondary antipyretic effect.

Most of these hypnotics have a tendency to lower the body temperature in small doses; but in large doses they produce a fall in temperature, not because of their nervous effect, but because they produce a weakening of the circulation and a weakening of the cardiac power.

Antipyrene has no effect on the temperature of normal animals, but on fevered animals it produces a lowering of temperature, probably by decreasing heat production and increasing heat dissipation. The decreased heat production is possibly brought about

by a stimulation of the thermolytic centre, thus decreasing heat production.

From this viewpoint it is possible to see that there is a comparison between the therapeutic action and their action on the catalysis of hydrogen dioxide.

ON THE PATHS OF ABSORPTION FROM THE LIVER.

By LAFAYETTE B. MENDEL and FRANK P. UNDERHILL.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

THE recent discussions of the pathogenesis of icterus have again raised the question as to the paths by which the bile constituents find their way into the blood in this pathological condition.[1] So long as the existence of perivascular lymph spaces or intralobular lymph vessels was denied or uncertain[2] the participation of the lymphatics in the pathological re-absorption and distribution of the bile constituents necessarily appeared unlikely. Under these circumstances it was assumed by some investigators that the excess of bile is discharged directly into the blood capillaries either through the liver cells themselves or through specially formed bile channels. Most histological writers now agree, however, in accepting the existence of perivascular lymph spaces or lymph channels as demonstrated.[3] With this anatomical feature established it was natural to attribute to the lymphatics the function of transporting the resorbed bile. The lymphatic path applied equally well whether the theory of interlobular absorption (Heidenhain) was accepted or the more probable explanation of Bürker,[4] by whom the path of absorption has been referred to the intralobular portions of the liver. In concluding a review of the literature Bürker writes (p. 276): "Wägt man die diesbezüglichen Arbeiten kritisch gegen einander ab, so kommt man entschieden zu der Ansicht, dass bei Stauung die Galle, so lange die Lymphwege

[1] *Cf.* EPPINGER: Ziegler's Beiträge zur pathologischen Anatomie, 1902, xxxi, p. 230; 1903, xxxiii, p. 123; MINKOWSKI: Zeitschrift für klinische Medicin, 1904, lv, p. 34; GERHARDT: Münchener medicinische Wochenschrift, 1905, No. 19, p. 889.

[2] *Cf.* BROWICZ: Wiener klinische Wochenschrift, July, 1900, No. 35.

[3] *Cf.* v. EBNER: Kölliker's Handbuch der Gewebelehre des Menschen, 1899, p. 242; EPPINGER: *Loc. cit.*; MINKOWSKI: *Loc. cit.*; MALL: Johns Hopkins Hospital bulletin, 1901, xii, p. 146.

[4] BÜRKER: Archiv für die gesammte Physiologie, 1901, lxxxiii, p. 241.

offen stehen, zunächst in diese gelangt, um erst dann, wenn Hindernisse auf dem Lymphwege sich geltend machen, auch in die Blutgefässe überzutreten." Eppinger[1] has written: "Nach dieser literarischen Uebersicht komme ich zu dem Schlusse, dass in der Frage der Pathogenesis des Icterus der Schwerpunkt in der Erledigung des Umstandes liegt, ob die Galle per vias lymphaticas oder direkt ins Blut ergossen wird. Damit hängt allerdings die Beantwortung der schliesslich wichtigsten Frage, ob es intraacinöse bezw. perivasculäre Lymphräume in Leberläppchen giebt oder nicht, zusammen. Die Beantwortung dieser Frage erleichtert wesentlich die der ersten Frage."

The doubt regarding the existence of the perivascular lymph spaces being removed, it is not difficult, from purely histological considerations, to conceive of an escape of bile constituents into these lymph sheaths. Those who attempt to account for the phenomena of icterus on the ground of mechanical obstruction in the bile passages portray the distention and rupture of the minute bile capillaries resulting in a discharge of bile into these perivascular spaces. There is a growing number of investigators who, like Minkowski, favor the possibility of icterus per parapedesin incident to a disordered function of the hepatic cells which permit secretion to go on in an unusual and reversed direction. These writers likewise follow the resorbed bile into the lymph sheaths already described. From here the hepatic products are believed to be transported to the true lymphatic vessels, ultimately reaching the blood by way of the thoracic duct. No difficulty is placed in the way of such a transference by the recent demonstration that the tissue spaces are, in general, closed cavities and do not communicate directly with the larger lymphatic channels;[2] for the conditions pertaining in the case of the liver appear otherwise according to the researches of Mall.[3]

However easy it may be to follow the bile out of its accustomed channels into the perivascular spaces bordering upon the liver cells, the widely accepted assumption that in icterus the hepatic products reach the blood stream solely or in large measure through the ways of the larger lymph vessels demands more careful consideration. In the case under consideration the bile-laden tissue lymph bathes the

[1] EPPINGER: Ziegler's Beiträge zur pathologischen Anatomie, 1902, xxxi, p. 260.

[2] *Cf.* MACCALLUM: Johns Hopkins Hospital bulletin, 1903, xiv, p. 105.

[3] MALL: *Loc. cit.*

intrahepatic blood capillaries. The possibility of an absorption of foreign substances directly into the circulation through the walls of the blood vessels has received a tardy acceptance at the hands of physiologists, despite the classic demonstration of Magendie made many years ago. In recent years Asher,[1] Starling,[2] and Mendel,[3] among others,[4] have given what we regard as convincing evidence of the direct participation of the blood vessels in absorption from lymph spaces other than those concerned in the liver.

Minkowski[5] has offered an interesting critique of the current view which assigns a pre-eminent rôle to the lymph channels in the introduction of bile products into the blood. He writes: "Ja, wenn dem so wäre, wie sollen wir uns überhaupt die ganze Stoffwechselthätigkeit der Leber vorstellen? Führt denn nicht das Blut der Leber fortwährend Stoffe zu, die von den Leberzellen aufgenommen und verarbeitet werden, und geben nicht die Leberzellen fortwährend Stoffe an das Blut ab, die, wie z. B. der Zucker und der Harnstoff, nicht erst durch den Ductus thoracicus, sondern direct durch die Lebervenen in den Kreislauf gelangen? Sollen wir auch hier einen directen Stoffaustausch zwischen Blut und Leberzellen deshalb in Abrede stellen, weil das Capillarendothel von der Leberzelle durch eine Saftspalte getrennt ist? Dürfen wir uns denn überhaupt alle diese Vorgänge des Stoffaustausches so rein mechanisch vorstellen, dass wir erwarten dürften, sie direct anatomisch verfolgen zu können? . . . es scheint durchaus nicht bewiesen, dass ein Icterus nur so zur Stande kommen kann, dass die Galle einfach also solche in die Lymphgefässe und von da aus in das Blut einfliesst, dass nicht auch Gallenbestandtheile aus den Leberzellen durch das Blut, in gleicher Weise wie andere Stoffe direct resorbirt werden können."

It is not difficult to explain the persistence of the current views regarding the absorption of bile in the liver. They arise from experiments started in Ludwig's[6] laboratory which seem to have retained

[1] ASHER: Zeitschrift für Biologie, 1893, xxix, p. 247.

[2] STARLING and TUBBY: Journal of physiology, 1894, xvi, p. 140; STARLING: Journal of physiology, 1898, xxii, p. xxii.

[3] MENDEL: This journal, 1899, ii, p. 342.

[4] The literature is admirably discussed by MELTZER: American Medicine, 1904, July 23 (Harrington Lecture).

[5] MINKOWSKI: Zeitschrift für klinische Medicin, 1904, lv, p. 39.

[6] *Cf.* FLEISCHL: Berichte der Gesellschaft der Wissenschaften. Leipzig, Mathem.-phys. Classe, 1874, xxvi, p. 42; KUNKEL: *ibid.*, 1875, xxvii, p. 236; KUFFERATH: Archiv für Physiologie, 1880, p. 92; HARLEY: *ibid.*, 1893, p. 294.

a convincing and conspicuous place in the literature despite various contradictory investigations. The physiological studies indicated in particular that when the thoracic duct is ligated, or its contents conducted to the exterior through a fistula, ligation of the common bile duct is no longer followed by the symptoms of icterus — hence the conclusion that the bile elements are conducted into the circulation *exclusively* by lymphatic channels. The experiments thus suggesting a complete incapacity of the hepatic blood vessels to absorb bile constituents directly, have been carefully repeated and extended by Wertheimer and Lepage [1] with different results, indicating a direct participation of the hepatic capillaries. To these may be added various earlier experiments demonstrating the direct absorption of foreign substances from the bile passages, even when the lymphatics are excluded.[2] It is not easy to understand why these researches have received so little appreciation unless it is attributed to the prevalent hesitation to admit a capacity for absorption on the part of blood vessels, as already mentioned above. Bürker, for example, dismisses the contradictory evidence with the remark that "physiology stands here, as so often elsewhere, before one of the riddles of which nature has furnished so many." He inclines, however, to direct the weight of possible experimental error against the newer views (p. 256).

We desire to record a few of our own experiments bearing upon the problem discussed. Among other things, they were planned to throw some light upon the discrepancies found in the literature. Tobias,[3] for example, failed to find sodium iodide in either blood or urine when a 5 per cent solution of it was injected into the gall bladder after ligation of the common bile duct. This was the case whether the thoracic duct was ligated or not. Strychnine, atropine, and sodium ferrocyanide were absorbed under the same conditions, and the conclusion was reached that the paths of absorption in the liver vary with the nature of the material absorbed. The experiment upon which Bürker [4] has placed especial emphasis in demonstration of the superior rôle of the lymph vessels in this absorption has also been repeated. Bürker injected milk into the Ductus choledochus in

[1] WERTHEIMER and LEPAGE: Archives de physiologie, 1897, p. 363; 1898, p. 334; Journal de physiologie, 1899, p. 259.

[2] These are reviewed by WERTHEIMER and LEPAGE: *Loc. cit.*, 1897, and by BÜRKER: Archiv für die gesammte Physiologie, 1901, lxxxiii, p. 252.

[3] TOBIAS: Travaux de laboratoire de Léon Fredericq, 1893–1895, v, p. 97 (quoted from BÜRKER).

[4] BÜRKER: *Loc. cit.*, p. 274.

rabbits, and observed that the lymph vessels issuing from the Porta hepatis and the perihepatic lymph glands soon showed a characteristic whitish appearance. The failure of emulsified fat, as it exists in milk, to penetrate the capillary walls does not, in our opinion, conclusively demonstrate the inability of *dissolved* substances to be absorbed by these blood vessels. Indeed, it might be expected that the globules of the fat emulsion would preferably follow the lymph channels if these are open. This is precisely comparable with the transference of fat from the intestinal lacteals; yet no one argues against the permeability of the intestinal blood capillaries for other compounds.

The method used in our experiments is a familiar one. Canulas were inserted into both ureters of dogs for the immediate collection of urine, and the lymph from the left thoracic duct was collected from a fistula. Injections of warm solutions containing readily detectable substances were made from a burette into the common bile duct with the minimum pressure sufficing to induce a return flow of the liquid. The animals were anæsthetized with morphine and A. C. E. mixture. A preliminary period of observation was always taken to insure the existence of a satisfactory normal flow of bile, urine, and lymph, which was measured throughout the trials. Extracts from the protocols follow.

Injection of indigo carmine. — In a dog weighing 15.6 kilos a lymph flow of 9.6 c.c. per ten minutes was noted. From 11.55 to 12.31, 36 c.c. of a 1.5 per cent solution of indigo carmine containing 0.7 per cent sodium chloride were run into the bile duct. The blue color was first noted in the urine at 12.20–12.25; in the uniformly flowing lymph at 12.28. In order to detect traces of the pigment, the lymph samples were treated with alcohol in which the color is readily soluble. The blue color of the lymph grew deeper and then lighter until at 1.45 it had vanished again. The urine continued to contain pigment up to the end of the observations at 3.45.

At the end of the injection the burette was disconnected and the bile allowed to flow outward through a canula. Blue color had practically disappeared from the renewed secretion long before the urine manifested any diminution of the absorbed color.

Injection of potassium iodide. — A dog weighing 21 kilos and having a lymph flow of 4 c.c. in five minutes received 23 c.c. of a 1 per cent potassium iodide solution at 10.43–11.13. Iodide was readily detected in the urine at 11.08–11.13, the test with the lymph at this period being

uncertain. Gradually stronger reactions were obtained in the lymph, the urine always affording a more marked test.

Injection of potassium ferrocyanide and milk. — A dog weighing 16 kilos and having a lymph flow of 13 c.c. in ten minutes received at fairly low pressure 40 c.c. of a 2.6 per cent solution of potassium ferrocyanide dissolved in milk, in forty minutes (12.06–12.45). The iron reaction was obtained with the urine from each kidney at 12.38–12.40 and continued strong. The lymph flowing steadily until two o'clock failed to show more than an uncertain trace of iron.

Injection of iodide and milk. — **I.** A dog weighing 9.5 kilos and having a normal lymph flow of 3.7 c.c. per ten minutes received 23 c.c. of milk containing 1 per cent of potassium iodide. The injection was very slow, and considerable pressure was required to effect the flow. In this experiment iodide was found in the lymph sixty-five minutes after the injection was begun. *Iodide failed to appear in the urine* during the two hours of observation.

II. In another dog weighing 14 kilos and having a lymph flow of 4.8 c.c. per five minutes, an injection of 19 c.c. of diluted milk containing 2.5 per cent of sodium iodide was made at 12.26–1.40. No test for iodide could be obtained with the quantity injected. As the injection fluid would not flow, even under pressure, at 3.30 the burette was disconnected and the obstructed bile and milk allowed to discharge. A second injection of a pure 2 per cent solution of sodium iodide was now made. After 47 c.c. had been introduced, at 5.00 the lymph gave a strong reaction for iodide. *The urine failed to give the iodide reaction.*

III. A third trial on a dog weighing 12 kilos and having a lymph flow of only 1.3 c.c. per ten minutes, resulted otherwise. Since it was noted that injected milk may accumulate in considerable quantity in the gall bladder, the cystic duct was ligated in this animal, and then an injection of 38 c.c. of milk containing 2.5 per cent of sodium iodide was made at moderate pressure within forty minutes. Blood withdrawn five minutes later from an artery contained considerable iodide. The flow of lymph became accelerated to 4 c.c. per ten minutes, but contained no detectable amount of iodide until two hours later. By this time the iodide, which had been abundant in the urine throughout the trial, had practically disappeared from this secretion.

A review of the preceding experimental data indicates plainly that the hepatic blood capillaries form no exception to those found elsewhere in the body in their permeability for substances which find their way into the perivascular lymph spaces. Our experience is in accord with that of Wertheimer and Lepage. Neither do the experi-

ments presented offer any indication of a predominating rôle of the lymph channels in carrying away the resorbed materials. In the trials with milk, which afforded the only exceptions to this general experience, the lymph was never found visibly richer in emulsified fat after the injections. The presence of the insoluble globules appeared at times to interpose an obstacle to the absorption of the soluble constituents, perhaps by obstructing the usual paths through the secreting hepatic tissue to the perivascular spaces. Such experiments with emulsions are, however, not strictly comparable with those in which bile itself is returned into the liver tissue. Experiments with obstructed thoracic ducts are likewise open to serious objection, since the conditions produced thereby in the lymphatic spaces must be distinctly abnormal. Finally, it is not impossible that the foreign substances found tardily in the lymph of the thoracic duct are at times derived indirectly by a subsequent transference from the blood vessels, and thus have a twofold source.[1]

The theory of absorption by the blood vessels has not entered into our discussion. We have aimed merely to emphasize that the possibilities of absorption from lymph spaces are not fundamentally different in the liver from those pertaining in the serous cavities and other lymph spaces.

[1] *Cf.* MENDEL: This journal, 1899, ii, p. 349.

A STUDY OF THE PROTEINS OF THE CASTOR BEAN, WITH SPECIAL REFERENCE TO THE ISOLATION OF RICIN.

By THOMAS B. OSBORNE, LAFAYETTE B. MENDEL, and ISAAC F. HARRIS.

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ALTHOUGH the proteins of the castor bean have been investigated by Ritthausen [1] and by Vines,[2] we have little definite information regarding them beyond the fact that this seed contains some proteose and a considerable proportion of a globulin which, both in composition and properties, closely resembles edestin from the hemp seed, and, like it, also crystallizes in octahedra. This globulin was first described in its crystalline condition by Ritthausen,[3] and later by one of us.[4] Respecting the other proteins nothing of importance is known.

The name *ricin* was given in 1889, by Stillmark,[5] to the highly toxic substance which he separated under Kobert's guidance, by the methods of protein separation then in use, and which he accordingly regarded as a globulin. Among subsequent investigators, Jacoby,[6] in particular, has questioned the protein nature of the poison. His opinion is based upon the failure, under the conditions of his experiments, to destroy the toxic properties of his ricin solutions by digestion with trypsin. Jacoby's results, which will be considered in greater detail further on, have given rise to a current view that the

[1] RITTHAUSEN: Archiv für die gesammte Physiologie, 1879, xix, p. 15; also Journal für praktische Chemie, 1881, xxiii, p. 481.

[2] VINES: Proceedings of the Royal Society, 1879–80, xxx, p. 387.

[3] RITTHAUSEN: Journal für praktische Chemie, 1879, xxiii, p. 481.

[4] OSBORNE: American chemical journal, 1892, xiv, p. 662.

[5] STILLMARK: Arbeiten des pharmakologischen Instituts zu Dorpat, 1889, iii, p. 59.

[6] JACOBY: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvi, p. 28.

toxine of the castor bean is a highly complex compound, although not a protein in the ordinary sense.[1] We have, accordingly, undertaken a study of the seed in order to determine more definitely the nature of its protein constituents, and to discover, if possible, with which, if any, of these proteins the toxic properties assigned to ricin are associated.

We have found that this seed contains proteins of the same character as the other oil seeds which have been examined, namely:

First, a considerable quantity of a globulin which can be obtained in octahedral crystals.

Second, a much smaller quantity of an albumin, coagulating at about 60° to 70°, the temperature at which it separates depending to a large extent on the rate of heating and other conditions, but in any case coagulating slowly and completely only after long heating.

Third, proteoses which appear to belong to several of the now recognized groups of this class of substances.

The toxic action was found to belong wholly to the preparations containing the coagulable protein, and never to be associated with preparations free from this albumin. The toxicity of the products consisting chiefly of albumin was extremely great, the most active preparation obtained by us proving fatal when administered subcutaneously to rabbits in the astonishingly small dose of 0.0005 milligram per kilo. In consequence of this extremely powerful physiological action many of the difficulties encountered by others in the preparation of this substance are explained, and much of the confusion that exists in the literature is cleared up. In view of the results of our work the more recent literature[2] can be discussed.

Dixson[3] found that a more active ricin could be obtained by precipitation with alcohol than by neutralizing a hydrochloric acid extract of the seeds with sodium carbonate, but that repeated precipitation with alcohol led to inactive preparations. Our experience has shown that the albumin is gradually, but pretty rapidly, coagulated by thus repeatedly precipitating with alcohol, so that a large part, if not all, may soon be separated from its solution in an altered form, while at the same time a larger or smaller amount of non-toxic

[1] OPPENHEIMER: Toxine und Antitoxine, 1904, p. 162.

[2] The references to the literature on ricin have been collected by JACOBY, Ueber Phytotoxine, Biochemisches Centralblatt, 1903, i, p. 289; OPPENHEIMER, Toxine und Antitoxine, Jena, 1904.

[3] DIXSON: Australian medical gazette, April, 1887, p. 156.

proteose, according to circumstances, is precipitated by the alcohol. The protein, thus treated, loses its toxicity, although the precipitate still consists of protein substance.

Stillmark[1] extracted castor beans with 10 per cent sodium chloride solution, saturated the extract with both magnesium and sodium sulphates, and then removed the salts from the precipitate by dialysis. Our experience shows that this process failed to separate the non-toxic globulin from the ricin, since the former is thus precipitated; and as its proportion, in the sodium chloride extract, is many times greater than that of the albumin, the product which Stillmark obtained must have contained but a small percentage of the toxic albumin. Nevertheless, Stillmark considered his product to be pure, and named it ricin.

Cushny[2] next investigated this problem, directing his attention particularly to the isolation of the ricin and determining its nature. Cushny's experience with alcohol precipitation has been confirmed by our own, and needs no further consideration. He seems to have been the first to realize adequately the great toxicity of this substance, and also the fact that a solution which showed strong toxic effects might be too dilute to respond to the ordinary tests for proteins. He found that, by concentration, such solutions gave protein reactions, and states that "so long as a solution is toxic it contains protein; as soon as the latter is removed the toxic action vanishes." Although Cushny regards the deadly dose of his ricin as vanishingly small, his minimal dose of 0.04 milligram per kilo is nearly one hundred times greater than ours. In order to obtain "pure ricin" Cushny extracted ricinus press cake (with what solvent he does not state), and saturated the solution with magnesium sulphate. As this salt precipitates the globulin as well as the albumin, the nature of the product obtained would depend much on whether the extract was made with water or salt solution.

The precipitate was redissolved by dialyzing into water, and, after filtering, yielded a yellowish solution "which, apart from salts, contained only a little organic matter, besides ricin."

Our experience with the coagulation of the albumin agrees well with that of Cushny, the temperature at which the coagulum separated being much influenced by the rate of heating.

[1] STILLMARK: Arbeiten des pharmakologischen Instituts zu Dorpat, 1889, iii, p. 59.

[2] CUSHNY: Archiv für experimentelle Pathologie und Pharmakologie, 1898, xli, p. 439.

In regard to the precipitation of the toxine with every precipitate produced in its solutions, especially those of other proteins, our experience has shown that this does not necessarily take place to the extent that Cushny's statements would lead one to believe. We succeeded in separating the globulin from all but minute traces of ricin, although it was thrown out of solutions containing much of this toxine. It is not improbable that this clinging of ricin to other precipitates is a case of adsorption, and that it does not occur to any greater extent than with many other bodies. That ricin can be separated from proteose by saturating the solution with magnesium sulphate, as Cushny states, we have also confirmed, as well as the fact that the proteose diffuses through the parchment membrane with relative ease. This proteose Cushny found to be non-toxic and devoid of agglutinating properties.

Müller[1] likewise failed to accomplish the separation of the toxine from protein. He was unable to destroy the toxic properties completely by peptic or tryptic digestion, and believed that they were independent of the protein molecule.

Jacoby[2] next made a study of ricin, using Merck's preparation, made according to Kobert's directions, as follows: The seeds were extracted with ether and with alcohol, and then with 11 per cent sodium chloride solution at 37°–40°. The filtered extract was next saturated with ammonium sulphate, and the precipitate dried at room temperature. It is evident that, besides the inorganic salts, the material, thus prepared, must have contained large amounts of both the globulin and the protein, and that only a very small proportion of ricin was present in it. Jacoby's experiments were mostly made with 1 gm. or less of this material, and therefore involved the use of very little of the toxic substance. Jacoby undertook to show that ricin was not destroyed by trypsin, by adding to his solutions, containing extremely small amounts of the pure toxine, 100 c.c. of trypsin solution.

Experiments with such very small quantities of ricin in the presence of such relatively very great quantities of other substances, mostly of unknown nature, are hardly to be accepted as conclusive, and therefore Jacoby's view, that ricin is not a protein because it is, sup-

[1] MÜLLER: Archiv für experimentelle Pathologie und Pharmakologie, 1899, xlii, p. 302.

[2] JACOBY: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvi, p. 28.

posedly, unaffected by trypsin, cannot be regarded as proved until it is confirmed by further experiments with larger quantities of ricin under more definitely known conditions.

Following Jacoby's example, Brieger[1] has attempted to separate the toxic element from the protein component of ricin preparations by digestion with papaïn (papayotin), but without success. He failed to obtain a protein-free ricin preparation which was pure in the chemical sense and still undiminished in toxic power. Further failures followed the attempt to make such a separation by the use of proteolytic bacteria (typhoid, cholera, etc.). Even after prolonged digestion the toxic properties remained unimpaired; but the proteid reactions were likewise still plainly in evidence. It is to be noted that Brieger succeeded in purifying commercial ricin preparations so that he obtained fatal results very speedily in rabbits with 0.5 mgr. per kilo of body weight, and readily with 0.01 mgr.

Experimental Part.

The protein constituents of the castor bean. — The castor beans employed were freshly gathered, and belonged to the cultivated variety known as *Ricinus zanzibarensis*. These were selected on account of their large size, which made it easier to remove the outer shell by hand.

Extraction a. — Nine hundred grams of these beans were thus freed from their shells,[2] crushed in a drug press, and treated with ether until most of the oil had been removed. The nearly oil-free meal was then extracted with 4 litres of 10 per cent sodium chloride solution, the extract filtered perfectly clear and dialyzed in running water for sixty hours. A large precipitate, A, separated, consisting of spheroids and crystals, which was filtered from the solution B.

Precipitate A was dissolved in 10 per cent sodium chloride solution, in which practically all was soluble, and the solution, which was distinctly alkaline to litmus, was saturated with sodium chloride. The precipitate that formed was filtered out and the filtrate dialyzed until all the globulin was precipitated. This was filtered out, washed with water and alcohol, and dried over H_2SO_4, giving Preparation 1, weighing 12.8 gm.

[1] Brieger: Festschrift für Robert Koch, Jena, 1903, p. 448.

[2] On account of the danger of poisoning, we would not advise this method of separating the shells unless it is done with rubber gloves.

The precipitate produced by saturating with sodium chloride was dissolved in a dilute solution of this salt, again thrown down by saturating with sodium chloride, and the filtrate treated in the way just described for Preparation 1. This yielded Preparation 2, weighing 13 gm. By repeating this process with the second precipitate; produced by saturating with salt, Preparation 3 was obtained, weighing 6.2 gm., and by dissolving in dilute brine the third precipitate, produced by saturating with sodium chloride, and dialyzing the filtered solution, Preparation 4, weighing 8.67 gm., was obtained. From these results it would appear that, although this globulin is precipitated by saturating with sodium chloride, a considerable proportion, each time, remains dissolved in the saturated salt solution owing to its solubility therein, which, while less than that in a 10 per cent solution of this salt, is still considerable in a saturated brine. Our former investigations have shown that there is no reason to believe that two globulins occur, and our present conclusion, that this globulin has a limited solubility in a saturated sodium chloride solution, seems to be therefore justified.[1] The total globulin thus obtained from this extract was 40.67 gm.

Solution B was saturated with ammonium sulphate, in order to bring the proteins which it contained into a smaller bulk, and the resulting precipitate was treated with a limited quantity of water, sufficient to dissolve only a part of it. The undissolved part, B a, was filtered from the solution, B b. The residue B a was then treated with more water, and the part still remaining undissolved was filtered out, washed and dried, giving Preparation 5, which weighed 3.7 gm. The filtrate from 5 was dialyzed in running water for twelve days, and then filtered from a small precipitate which, washed and dried, formed Preparation 6, weighing 0.52 gm. This preparation, which separated on dialysis, contained but an insignificant amount of matter soluble in 5 per cent sodium chloride, and we have therefore no conclusive evidence that this substance, separating during the dialysis, was originally a globulin; we shall, in fact, give reasons why it is much more probable that it was an altered or albuminate form derived from the albumin. Albumins which behave in this way are present in nearly all seeds.

The filtrate from 6 was dialyzed into alcohol, until concentrated to

[1] The distinction formerly made between myosins and vitellins, based on precipitation by saturating with sodium chloride, cannot be applied to the vegetable globulins.

a small volume, and then filtered from a considerable precipitate, which, when dried, weighed 3.84 gm. This substance was treated with water, and the part that did not dissolve was filtered out, washed and dried, giving Preparation 7, weighing 1.45 gm.

Solution B b, above mentioned (p. 264), was dialyzed for twelve days in running water, and then filtered from a small amount of insoluble matter, which, when washed and dried, formed Preparation 8, weighing 2.2 gm.

The filtrate from 8 was dialyzed into alcohol, until concentrated to a small volume, when it was filtered, and the precipitate treated with absolute alcohol, dried, and found to weigh 20.7 gm. This latter was then extracted with water, and the substance insoluble therein was filtered out, washed, and dried, giving Preparation 9, weighing 3.07 gm.

The filtrate from 9 was united with that similarly produced from 7, and the two saturated with magnesium sulphate. The precipitate which resulted was redissolved in water and again saturated with magnesium sulphate. The second precipitate, thus obtained, was freed from solution by spreading on a porous plate, dissolved in about 25 c.c. of water, and the solution dialyzed in running water for three days. The clear solution was then evaporated over sulphuric acid in a vacuum, and the residue was scraped from the dish and found to weigh 1.39 gm. (Preparation 10). As this substance was found to be exceedingly toxic, and constituted the purest ricin made in this experiment, it was necessary to use the utmost caution in removing it from the dish in which it had dried down. This substance will be described in detail later.

The filtrates from the two precipitations with magnesium sulphate were united, dialyzed in running water for nineteen days and then into alcohol, until all the protein had separated. The precipitate thus produced was filtered out, dissolved in water, and again thrown down by pouring into a large volume of alcohol.

The precipitate that separated was washed with alcohol and dried over sulphuric acid, giving Preparation 11, weighing 4.08 gm. It consisted of proteose, and had no toxic action. This proteose represented the water-soluble substance recovered from two products, weighing respectively 20.7 and 3.84 gm. From this we removed Preparations 7, 9, and 10, whose aggregate weight was 5.92 gm., leaving 18.62 gm. of proteose, of which only 4.08 gm. were finally recovered, or only about 22 per cent. This loss, we think, is chiefly due

to the diffusion of the proteose that occurred during the long dialysis required for its isolation.

Several of these preparations were analyzed, in order to determine something of their relations, which might serve as a guide in future attempts to prepare ricin.

	6	7	8	9	10		11	
					I	II	I	II
Carbon	....	52.20	....	51.90	47.74	47.52	43.78	43.85
Hydrogen	....	6.99	....	6.87	6.58	6.61	6.17	6.32
Nitrogen	14.84	15.65	15.62	15.85	14.03	....	17.19	....
Ash	0.63	0.16	1.17	0.13	2.84	....	3.77	....

The average, ash-free, composition shown by these analyses was the following:

	6	7	8	9	10	11
Carbon	....	52.28	....	51.96	49.03	45.54
Hydrogen	....	7.00	....	6.87	6.60	6.49
Nitrogen	14.93	15.68	15.81	15.87	14.44	17.86

Of these preparations 6, 7, 8, and 9 represent insoluble or albuminate products, formed during the process employed for their isolation. These are much alike in composition, and doubtless consist mostly of derivatives of the coagulable protein contained in this extract, for, with the removal of this insoluble matter, the proportion of the albumin evidently diminished. The relations of the figures found for Preparation 10 are not apparent, especially that for nitrogen, which we have reason to believe is found too low, probably owing to an analytical error. Unfortunately this determination cannot be repeated, as no more of the substance exists. This Preparation 10 contained a large proportion of the protein, coagulating on long heating at about 70°, but owing to the small amount of the preparation, the proportion was not determined. Its toxicity was high, 0.002 of a milligram per kilo, when subcutaneously injected into rabbits, proving fatal with all the characteristic symptoms of ricin poisoning.

The proteose, Preparation 11, was wholly free from toxicity, in agreement with Cushny's observations, and likewise with his statement that the toxine is completely precipitated by saturating with magnesium sulphate.

Extraction b. — Since it seems highly improbable that the proteoses could have yielded the insoluble albuminate-like products obtained from this extract, we had little doubt that these were derivatives of the albumin, and that if this were in fact so, they possibly repre-

sented alteration products of ricin. With this in view we undertook another extraction of *Ricinus* seeds, of the same variety as used in Extraction a, employing fractional precipitation with ammonium sulphate, hoping thereby to avoid the alteration of the albumin which took place to such an extent in the presence of alcohol. In this extraction the shells were not removed, the entire seed being crushed and the oil extracted with ether. Of the meal thus prepared 1305 gm. were extracted with 6 litres of 10 per cent sodium chloride solution, the extract filtered perfectly clear, and dialyzed for four days in running water. The globulin which was thus precipitated, when washed and dried in the usual manner, weighed 190 gm. In the filtrate, which measured 14,750 c.c., we dissolved 5044 gm. of ammonium sulphate, which is equal to approximately 45 per cent of the amount required for complete saturation with this salt. The precipitate, I, which separated, was filtered out, and 1680 gm. more of the sulphate were added to the filtrate, raising its quantity to 60 per cent of complete saturation. Precipitate II, which separated, was filtered out, and 1122 gm. more sulphate added to the filtrate, making it 70 per cent saturated and producing Precipitate III. The filtrate from III, when fully saturated, yielded Precipitate IV. An examination of these four fractions showed that I contained much that was coagulated by heating below 80°, whereas II and III contained very little of the albumin, and a considerable quantity of substance that, like a heteroproteose, separated on cooling after the solution had been heated for some time in a boiling-water bath. Precipitate I differed from the others in that it was a flocculent, voluminous product, which showed no tendency to unite to a dense, gummy mass, as did the others. In this respect these latter resembled the ordinary proteoses produced by peptic digestion. Precipitate IV contained no albumin or heteroproteose.

These four fractions were then treated as follows. Precipitate I was dissolved in water, the solution filtered clear, and, in order to bring it into a smaller volume of water, it was precipitated by saturation with ammonium sulphate. The precipitate (I a) was sucked out very dry, dissolved in 1000 c.c. of water, and precipitated by an equal volume of saturated sulphate solution. The precipitate (I b) was sucked dry, dissolved in 1000 c.c. of water, and saturated sulphate solution added until precipitation began. This required 250 c.c. of saturated sulphate, corresponding to one-fifth of complete saturation. The saturation was then raised to one-third by adding 250 c.c. more

sulphate solution, and the precipitate (I c) produced was filtered out, dissolved in 250 c.c. of water, and the solution dialyzed for eleven days in running water. A precipitate of globulin, which had separated, was filtered out, and, when washed and dried over sulphuric acid, gave Preparation 12, weighing 5.74 gm. The clear filtrate from 12 was evaporated on a plate at 50°, and, as this was presumably very toxic, enough petroleum benzine was poured over it to completely cover all the substance and prevent its flying about when scraped from the plate. The latter operation was done out of doors, a sheet of glass being put over the plate, under which the scraping was done. The residue was then transferred to a bottle, the benzine driven off at a low temperature, and the residue dried. This formed Preparation 13, which weighed 11.93 gm.

The filtrate from I b was raised to one-half saturation by adding 500 c.c. of saturated sulphate, and the precipitate (I d) produced was sucked out. This, together with precipitate I x c, soon to be described, was dissolved in a little water, and its solution dialyzed for eleven days. A small precipitate of globulin, together with considerable insoluble albuminate which had formed, was filtered out, giving Preparation 14, weighing 1.35 gm., and the clear filtrate, evaporated at 50° and treated in the same way as Preparation 13 had been. This preparation, 15, weighed 23.89 gm.

The filtrate from precipitate I d was saturated with ammonium sulphate, giving precipitate I x b, which was united with I x a, sucked dry, dissolved in water, and a saturated solution of ammonium sulphate added until a considerable precipitate, I x c, had formed. This was dissolved in water and added to precipitate I d, as mentioned just above. We now consider that we made a mistake in uniting these, but were led to do so from the fact that this precipitate contained much albumin; it being the plan of our experiment to bring together, as far as we could, all of the albumin, as free as possible from other proteins. The filtrate from this partial precipitation, produced by the sulphate solution, was brought to complete saturation, the resulting precipitate (I e) dissolved in water, its solution dialyzed for seventeen days in running water, filtered from a very slight precipitate of globulin, and the clear solution evaporated and treated in the way described for preparing 13. The product thus obtained weighed 8.75 gm., and formed Preparation 16.

Precipitate II, from the original extract, was dissolved in water, and its solution dialyzed till nearly free from sulphate. A trace of

insoluble matter resulted, from which the solution was filtered. A small part of this solution, when heated for some time in a boiling-water bath, gave a very small coagulum, and, when this was filtered out, a considerable precipitate on cooling, which dissolved on heating and reappeared on cooling, after the manner of a heteroproteose. The rest of the solution was evaporated at 50°, and left a residue weighing 8.73 gm., which formed Preparation 17.

Precipitate III was dissolved in water, and in order to reduce the volume of its solution it was reprecipitated by saturating with ammonium sulphate. This last precipitate was then dissolved in the least possible quantity of water, and its solution dialyzed for seventeen days. A very little substance separated, which, however, did not appear to be a heteroproteose, as it did not give the heat reaction. It had all the properties of a globulin, and was coagulated by heat. There was too little of this substance for any further examination. The clear solution, from which this globulin had separated, was evaporated at 50°, and 2.35 gm. of Preparation 18 obtained.

Precipitate IV was dissolved in water and twice precipitated by pouring its solution into alcohol. It was then dissolved in water, dialyzed nine days in water, and then in alcohol, until the dissolved proteose was precipitated, when the latter was dehydrated with absolute alcohol and dried over sulphuric acid. This gave Preparation 19, weighing 11 gm. This preparation contained no coagulable protein, nor any of the heteroproteose-like substance. The proteose constituting this preparation was relatively diffusible, and much was lost by dialyzing its solution into water.

The general method followed in fractioning the protein constituents of this extraction is shown by the scheme on the next page.

These same preparations were dried at 110° and analyzed with the following results:

	13	15	16	17	18	19
Carbon	50.76	50.22	48.52	48.29	48.85	47.56
Hydrogen	6.87	6.85	6.59	6.60	6.64	6.15
Nitrogen	16.52	16.85	18.60	18.22	18.57	18.68
Sulphur	1.67	1.93	2.84	2.98	2.70	2.81
$(NH_4)_2SO_4$	1.69	1.61	0.54	1.36	0.25	0.91
Ash	0.71	0.70	0.43	0.98	0.23	0.84

These analyses, calculated ammonium sulphate and ash free, after deducting the nitrogen, sulphur, and hydrogen of the latter, gave the following figures for the composition of the organic part of these preparations:

EXTRACTION B.

1305 GM. RICINUS SEEDS + NaCl SOL. EXTRACT DIALYZED 4 DAYS.

Pp. Globulin, 190 gm. | Sol. = 14,750 c.c. + 5044 gm. $(NH_4)_2SO_4$ = 45% saturation

Pp. I. | Sol. + 1680 gm. $(NH_4)_2SO_4$ = 60% saturation

Pp. II. | Sol. + 1122 gm. $(NH_4)_2SO_4$ = 70% saturation

Pp. III. | Sol. + 2500 gm $(NH_4)_2SO_4$ = saturation

Pp. IV. | Sol. contained no more protein

Pp. I + H_2O = Sol. + $(NH_4)_2SO_4$ to saturation.

Pp. I.a + 1 litre H_2O = Sol. + 1 litre saturated $(NH_4)_2SO_4$ sol. = ½ saturation | Sol. contained no protein

Pp. I.b + 1 litre H_2O = sol. + 250 c.c. saturated $(NH_4)_2SO_4$ sol. = ½ saturation = incipient precipitation + 250 c.c. more $(NH_4)_2SO_4$ sol. = ½ saturation | Sol. + $(NH_4)_2SO_4$ to saturation

Pp. I.c + 250 c.c. H_2O = sol. Dialyzed 11 days | Sol. + 500 c.c. saturated $(NH_4)_2SO_4$ sol. = ½ saturation | Pp. I.x a + H_2O = sol. + $(NH_4)_2SO_4$ till considerable pp. formed | Sol. contained no protein

Prep. No. 12. 5.74 gm. | Sol. evaporated. Prep No. 13. 11.93 gm. ricin | Pp. I.d + I.x c + H_2O = sol. Dialyzed 11 days | Sol. saturated with $(NH_4)_2SO_4$ | Pp. I.x c added to Pp. I.d | Sol. saturated with $(NH_4)_2SO_4$

Pp. Prep. No. 14. 1.35 gm. | Sol. evaporated. Prep. No. 15. 23.89 gm. ricin | Pp. I.x b united with I.x a | Sol. contained no protein | Pp. I.e + H_2O = sol. Dialyzed 17 days | Sol contained no protein

Pp. II + H_2O = sol, dialyzed

Pp. II.a very small | Sol. evaporated = Prep. No. 17. 8.73 gm.

Pp. III + H_2O = sol. saturated with $(NH_4)_2SO_4$

Pp. + H_2O = sol. dialyzed 17 days | Sol. contained no protein

Pp. very small | Sol. evaporated = Prep. No. 18. 2.35 gm.

Pp. IV + H_2O = sol., poured into alcohol

Pp. + H_2O = sol. + 3 vols. alcohol | Sol.

Pp. + H_2O = sol. dialyzed 9 days in water, then in alcohol

Pp. = Prep. 19. 11 gm. | Sol.

Pp. very little | Sol. evaporated. Prep. No. 16. 8.75 gm.

	13	15	16	17	18	19
Carbon	52.01	51.41	49.00	49.44	49.10	48.41
Hydrogen	7.02	7.00	6.65	6.76	6.68	6.26
Nitrogen	16.56	16.90	18.67	18.37	18.63	18.82
Sulphur	1.29	1.77	2.74	2.70	2.73	2.63
Oxygen	23.12	22.92	22.94	22.83	22.87	23.88
	100.00	100.00	100.00	100.00	100.00	100.00

Of these six preparations 16, 17, 18, and 19 consisted almost wholly of proteose, while 13 and 15 were mixtures of both albumin and proteose. In these two latter preparations the chief interest centres, since they contained nearly all the toxic substance of the extract, the other preparations being practically destitute of any toxic effect. For rabbits the minimal fatal dose of 13 was 0.0005 mgm. per kilo, when subcutaneously injected; that of 15 about 0.001 mgm. per kilo.

The amount of coagulable albumin in these two preparations was determined by dissolving 0.3083 gm. of 13 and 0.4070 gm. of 15 in 5 per cent sodium chloride solution, and heating for an hour at 95°. The coagulum was filtered from each, washed and dried to constant weight at 110°. That from 13 weighed 0.2178 gm., equivalent to 70.64 per cent; that from 15 weighed 0.1718 gm., or 46.48 per cent. The nitrogen in these coagula was determined by Kjeldahl as 15.8 and 16.0 per cent of the respective coagulum, which agrees closely with that found in our preparations 7, 8, and 9. The specific rotation of 13 was $(a)_D - 28.85°$; of 15, $-30.2°$.

The proportion of the different forms of nitrogen in 13 was found, according to Hausmann's modified method,[1] to be as follows:

Nitrogen as ammonia	1.74%
Basic nitrogen	4.29%
Amino nitrogen	10.42%

Neither preparation contained any phosphorus.

If the composition of mixtures of the coagulated albumin, C 52.12; H 6.93; N 15.78 per cent, and the proteose preparation 16, C 49.00; H 6.65; N 18.67 per cent, are calculated, we have for 70.64 per cent albumin and 29.36 per cent proteose C 51.21; H 6.85; N 16.63; found C 52.01; H 7.02; N 16.56; and for a mixture of 46.48 per cent albumin and 53.52 per cent proteose C 50.45; H 6.78; N 17.32, found C 51.41; H 7.00; N 16.90 per cent. The results thus obtained show

[1] OSBORNE and HARRIS: Journal of the American Chemical Society. 1903. XXV, p. 323.

that both preparations contained little else than protein matter, and that 13 contained at least 70.64 per cent of coagulable albumin, and that 15 contained 46.48 per cent. As no preparations, which we have obtained, showed toxic properties when free from albumin, and as 13, containing much more albumin than 15, was decidedly the more toxic, it seems to us that it is almost certain that ricin and the albumin are one and the same substance. In what respect this toxic albumin differs from the other non-poisonous proteins our analyses and examination of these preparations give no hint. So far as our determinations go, ricin does not differ from ordinary proteins in composition, heat coagulation, color reactions, precipitation reactions, specific rotation, or in the state of combination of its nitrogen.

Preparations obtained by the methods of desiccation outlined above dissolve perfectly in water or " physiological saline " to form an absolutely clear and colorless solution. The dosage is thus easily regulated. Specimens preserved many months have shown no diminution in physiological activity such as has been reported by some investigators for commercial ricin preparations.

In view of the extremely small proportion of ricin required to produce profound physiological changes resulting in death after the lapse of a considerable interval of time, it seems to us to be probable that it acts as an enzyme. If this supposition be true, which is also supported by many of the other properties of ricin, notably the effect of heat, we have in these results strong evidence of the protein nature of enzymes.

Physiological experiments. — The study of the proteins of the castor bean has involved observations on their physiological behavior in a variety of reactions which have been more or less carefully investigated by previous workers. Aside from its pronounced toxicity, resulting in death with typical pathological findings, ricin is capable of producing a marked agglutination and sedimentation of red blood corpuscles in vitro. Furthermore, it has aroused especial interest in recent years in view of the studies of Ehrlich,[1] who succeeded, by varying methods of preliminary treatment, in establishing a high degree of immunity to the castor bean toxine, and in demonstrating the production of an antitoxic substance, antiricin, in the blood of the immunized animals. Our investigation of the physiological be-

[1] Ehrlich: Deutsche medizinische Wochenschrift, 1891, p. 976; p. 1218; Fortschritte der Medizin, 1897, p. 41.

havior of the protein preparations from the castor bean has involved the study of their toxic and agglutinating properties in particular.

Relative toxicity of the castor bean protein preparations. — Most of our experiments on the toxicity of the various preparations described in the earlier part of this paper have been made on rabbits and guinea-pigs, the former being especially susceptible to the action of the poison. A few trials were also made with cats, dogs, and frogs. The toxicity of ricin for most of the familiar laboratory animals has long been known. Unless otherwise stated, the substance to be investigated in our experiments was introduced into the body subcutaneously, with due antiseptic precautions.

In order to form some idea of the relative toxicity of the products examined, it is of interest to compare the fatal doses given by previous investigators in cases of acute intoxication resulting in death within a brief period, usually twenty-four to thirty-six hours. Ehrlich produced death with 0.03 mgm. per kilo of body weight. Cushny[1] obtained speedy lethal effects in rabbits with 0.04 mgm. Müller[2] and Jacoby[3] produced death without fail within thirty hours with 0.5–0.6 mgm. of Merck's ricin or their "purified" ricin. With a dose of 0.008 mgm. of Merck's ricin, administered to a guinea-pig (and therefore equivalent to 0.02–0.03 mgm. per kilo), Flexner[4] noted a survival of the animal for six weeks. Finally, Brieger[5] produced death in rabbits with 0.01 mgm. per kilo with a partly purified preparation. Few known compounds approach ricin in exhibiting so high a degree of toxicity. Among them snake venoms are conspicuous, 0.079 mgm. of cobra poison per kilo of animal sufficing according to Martin,[6] and 0.02–0.04 mgm. of the venom of the Hydrophidæ, according to Rogers,[7] to produce lethal effects.

The symptoms which accompany ricin intoxication, together with the pathological changes produced in the tissues by the poison, have been described in detail by various writers.[8] The appearance of

[1] Cushny: Archiv für experimentelle Pathologie und Pharmakologie, 1898, xli, p. 439.

[2] Müller: *Ibid.*, 1899, xlii, p. 302.

[3] Jacoby: *Ibid.*, 1901, xlvi, p. 28.

[4] Flexner: Journal of experimental medicine, 1897, ii, p. 197.

[5] Brieger: Festschrift für Robert Koch, 1903, p. 445.

[6] Martin: Proceedings of the Royal Society of London, 1889, xlvi, p. 108.

[7] Rogers: *Ibid.*, 1903, lxxi, p. 481.

[8] Flexner: *Loc. cit.*; Müller, *Loc. cit.*; Oppenheimer: Toxine und Antitoxine, 1904, p. 166.

noticeable signs is comparatively slow, even when large doses are administered. The animals refuse to eat after a few hours; but the onset of severe symptoms is always rather sudden. Convulsions and opisthotonus are followed by extreme relaxation and again by a return of the convulsive effects. There are no truly specific symptoms to be noted, and the influence of variation in dosage is exhibited primarily in the shortening or prolongation of the latent period before the appearance of appreciable symptoms. In no instance have we seen death ensuing sooner than fifteen to eighteen hours after administration of the poison subcutaneously. Flexner reports the isolated case of death within six hours in a rabbit after a dose of three mgm. of ricin per kilo.

Among the gross pathological changes noted, the punctiform hæmorrhages in the peritoneal cavity scattered over the omentum and along the serous covering of the intestine were those most frequently observed. Other similar ecchymoses were often noted. Peyer's patches and the retroperitoneal lymph glands were usually, though not invariably, found somewhat swollen and markedly congested or hæmorrhagic. Not infrequently the peritoneal cavity contained a large excess of somewhat opaque fluid. Acute ulcerations of the intestine were never found. In this respect, as in other details, our findings at autopsy agree perfectly with the careful descriptions of Flexner.[1] This writer, in particular, has pointed out that the toxine of the castor bean exerts its deleterious action upon cells and tissues, much like the bacterial poisons. The destructive effects are evident, both at the point of entrance of the toxine, in the subcutaneous tissue, and at the places of exit, as along the stomach and intestinal wall. The elimination of the poison by the gut has been ascertained by several investigators.[2] The significance of this path of excretion has already been indicated by one of us in another connection;[3] and the passage of the poison through the wall of the intestinal tract undoubtedly accounts for the local histological lesions observed there. With respect to the pathological conditions produced at the site of injection, less uniformity of opinion appears to exist. We have therefore carefully noted the local effects in a large number of animals, and have uniformly found a reddening of the subcutaneous

[1] FLEXNER: *Loc. cit.*

[2] CUSHNY: *Loc. cit.*, p. 443; STEPANOFF: Annales de l'Institut Pasteur, 1896, x, p. 663.

[3] MENDEL and THACHER: This journal, 1904, xi, p. 15.

tissue which not infrequently presented a distinct hæmorrhagic appearance, and only occasionally a noticeable œdematous condition.[1] A noticeable necrosis was never obtained. These appearances were all missing when non-toxic proteins were injected subcutaneously. It has been suggested that the pathological changes, such as inflammation and necrosis, noted at the point of inoculation, may be attributable to contaminations introduced with the actual toxine;[2] our observations, however, lend no support to such a view.

The experiments with the various castor bean proteins, the preparation of which was described in the earlier part of this paper, at once showed that the toxic compound is present in the fractions representing the albumin constituent of the seed, and absent entirely, or practically so, from the globulin preparations. Thus in Extraction a, p. 263, all the fractions obtained from Solution B were toxic with the exception of Preparation 11, representing the proteoses of the seed. Since some of the fractions had become largely insoluble in the course of their preparation (owing to albuminate formation), it was difficult to secure an absolutely accurate dosage. Later we succeeded in obtaining all of the products unaltered and in perfectly soluble form. Preparation 10, the purest isolated in this series, was exceedingly toxic. A single detailed protocol will suffice to illustrate the character of all of these trials.

A rabbit weighing 1300 gm. received a subcutaneous injection of 1 c.c. of a solution of Preparation 10, containing 24 mgm. per litre. 1 c.c. = 0.024 mgm. or *0.019 mgm. per kilo of body weight.* On the following day the animal appeared sick and would not eat. During the first forty hours after the injection no further symptoms were noted; within six hours thereafter the rabbit died. The autopsy showed the characteristic pathological changes; subcutaneous tissue and underlying muscles hæmorrhagic at the seat of injection, not elsewhere; extensive ecchymoses on the omentum and outer layer of the cœcum; Peyer's patches were not swollen; the liver showed numerous light-colored spots; intestinal and mesenteric vessels distended.

A series of trials on rabbits with varying doses of Preparation 10 indicated the delay in the lethal outcome with diminishing quantities. The pathological findings were essentially similar in every instance. The precise time of death was, of course, rarely ascertained.

[1] Grateful acknowledgment is made of the assistance of Dr. R. F. Rand and Dr. F. P. Underhill in making some of the many autopsies.

[2] OPPENHEIMER: Toxine und Antitoxine, 1904, p. 165.

PREPARATION 10.

Dose per kilo.	Time of death.
0.31 mgm.	18 hours.
0.058 "	Within 48 hours.
0.04 "	" " "
0.019 "	46 hours.
0.0088 "	60 "
0.002 "	82 "

With these toxic effects, the observations made after injection of other fractions isolated from the same seeds may be contrasted. Where the preparations were not perfectly soluble, the amount actually dissolved was determined as accurately as possible in separate portions of the solutions. Rabbits were used.

Preparation.	Dose per kilo of body weight.	Observations.
	mgm.	
No. 4. Globulin	0.5	Animal remains apparently well.
No. 4. "	3.1	Do.
No. 4. "	8.5	Animal died in 5 days.
No. 3. "	1.48	Animal apparently well.
No. 3. "	5.5	Animal died after 9 days without typical symptoms.
No. 2. "	0.6	Animal remains apparently well.
No. 1. "	1.2	Do.
No. 11. Proteose	3.2	Do.
No. 8. Albuminate	0.5	Animal sick, but recovers.
Excelsin (globulin) from Brazil nut	2.7	Animal well.

The soluble parts of the other albumin-containing fractions 5, 7, and 9, were all extremely toxic in doses of 1–2 mgm. No attempt was made to determine the lower limits of toxicity.

In considering the fatal outcome noted above with large doses of two of the globulin fractions, the result is not surprising when the extreme toxicity of the active agent is considered. Thus a *very slight* adherence of the more soluble albumin fraction might easily render *larger* doses of globulin fatal. In comparison, however, such quantities lose their significance.

The toxic character of the albumin preparations from the castor bean, in contrast with the other proteins present, was shown even more strikingly with the products isolated in the second experiment (p. 266). The results of the trials on animals are summarized from the protocols in the next table.

These records clearly indicate the possibility of a fractional separation of the *ricin* which is found absent from the globulin and proteose fractions. Corresponding with the larger proportion of coagulable protein in 13 (70 per cent) in comparison with 15 (46 per cent), the former was found to be relatively more toxic as determined by the minimum lethal dose. On the other hand, injections of castor bean globulin in quantities equal to one hundred or more fatal doses of ricin failed to elicit toxic symptoms. The significance of the fatal effects sometimes produced by very large doses of intermediate fractions like 17 has already been commented upon.

The protocols, here reported, confirm the relatively greater susceptibility of the rabbit to ricin intoxication in comparison with guinea pigs. Cats are apparently still less sensitive, although we have not determined the minimum lethal dose for these animals. The experiments with cats and dogs were undertaken for the purpose of observing the gross pathological lesions produced; they were found to be essentially similar to those described above. Frogs were found to be peculiarly resistant to the action of ricin at the temperature of the cold tanks in which they were usually kept. Cushny[1] has called attention to the behavior of these animals to ricin, and states that they may live for long periods before death from this toxine intervenes. We failed to find toxic symptoms in frogs kept at low temperatures when less than 1.5 mgm. of the most active preparation, 13, were subcutaneously introduced. Doses of 5 to 8 mgm., however, killed animals of 34 to 40 gm. in from nine to eleven days. At room temperature, quantities as small as 2 mgm. proved fatal in one day, while 6 mgm. of Preparation 18 were without effect. When the aquarium was kept at somewhat higher temperature (25°–30°) 2 mgm. of Preparation 15 rapidly killed frogs of 60 gm. in weight, while control animals were not injured by the warmth applied. The resistance of this species is therefore apparently associated with the familiar retardation of metabolic processes at the lower temperatures.

Ricin immunity. — Ehrlich[2] succeeded in establishing an immu-

[1] CUSHNY: *Loc. cit.*, pp. 442 *et seq.*

[2] EHRLICH: *Loc. cit.*

nity towards the toxic effects of ricin preparations in white mice and rabbits, both by feeding and by subcutaneous administration of the

TOXICITY OF CASTOR BEAN PROTEINS.

Preparation used.	Animal.	Dose per kilo of body weight.	Effects noted.
		mgm.	
No. 13. Albumin 70%, proteose 30% . . .	Rabbit	0.1	Death in 1 day.
		0.0014	Death in 8 days.
		0.0005	Death in 7 days.
		0.00045	None.
		0.00027	None.
	Guinea-pig	0.015	Death in 8 days.
		0.0032	Death in 7 days.
		0.001	None.
No. 15. Albumin 46%, proteose 54% . . .	Rabbit	0.12	Death in 36 hours.
		0.1	Death in 21 hours.
		0.006	Recovery.
		0.0014	Death in 19 days.
	Guinea-pig	0.5	Death within 48 hours.
	Cat	0.1	Death in 5 days.
		0.1	Death in 2 days.
		0.1	Recovery (?)[1].
	Dog	0.5	Death within 48 hours.
No. 16. Proteose . . .	Rabbit	1.0	None.
	Guinea-pig	2.0	None.
No. 12. Globulin and albuminate	Rabbit	?	None.
	Guinea-pig	?	None.
No. 14. Globulin and albuminate	Rabbit	Less than 0.5[2]	Death within 4 days.
No. 17. Proteose with trace of albumin . .	Rabbit	2.2	Death in 1 day.
		0.21	None in 12 days.
	Guinea-pig	1.0	None in 12 days.
No. 18. Proteose . .	Rabbit	0.53	None.
		0.1	None.
	Guinea-pig	1.1	None in 12 days.
No. 19. Proteose . .	Rabbit	0.136	None in 12 days.
	Guinea-pig	1.1	None in 12 days.

[1] A large abscess developed at the seat of the injection, and the animal was killed after thirteen days, apparently improved.

[2] Owing to the insolubility of this preparation, no accurate dosage could be made.

toxine. In studying the toxidity of some of our preparations exhibited by way of the mouth, we have incidentally made a few observations bearing on the subject. Preparation 15 was used for the trials. Single doses of 40, 21, and 4 mgm. respectively administered in solution by means of a stomach tube each proved fatal to medium-sized rabbits in about two days. The pathological findings were typical. A single dose smaller than 4 mgm. failed to produce death. One animal received nearly 0.2 gm. in the course of about three months, beginning with 0.5 mgm. and increasing to 1, 5, 5, 10, 25, 20, 40, 32, 45 mgm. per dose at intervals of a few days. The rabbit remained well, and after an interval of a month showed no unfavorable response to repeated doses of 5 mgm. per os. Another animal less gradually immunized with a total ingestion of 62 mgm. succumbed to a following dose of 30 mgm. in six days.

The action of the castor bean protein preparations on blood. — The characteristic influence of ricin preparations on extravascular blood or suspensions of corpuscles is exhibited in the agglutination of the erythrocytes followed by the sedimentation of flocky masses upon the bottom of the retaining vessel. A clear, practically colorless serum is obtained in this way. The bloods from different species, in correspondence with our own observations, show different degrees of sensitiveness as they do toward bacterial lysins. Lau[1] has asserted that the corpuscles of fish blood are completely resistant to ricin; but Fraenkel's[2] observations on Barbus fluvialis suggest that the immunity is a relative one merely, since larger doses produce agglutination. Kobert[3] was unable to effect an agglutination of the corpuscles of Sipunculus with ricin, and concluded that their stroma must vary in composition from that of the vertebrates.

We have found an agglutinating power associated with all the distinctly toxic preparations from the castor bean, but not manifested by the globulin or proteose fractions or proteins from some other vegetable sources. Owing to the indefinite statements of most writers, it is impossible to institute quantitative comparisons as in the case of the toxic properties. In correspondence with the extremely marked toxicity of our active preparations, the agglutinating power was likewise very high. The method of making comparative observations was as follows:

[1] Lau: Dissertation, Rostock, 1901.

[2] Fraenkel: Beiträge zur chemischen Physiologie, 1903, iv, p. 224.

[3] Kobert: Archiv für die gesammte Physiologie, 1903, xcviii, p. 411.

The preparation to be tested was dissolved in isotonic salt solution (0.92 per cent sodium chloride) or 10 per cent sodium chloride solution, a measured quantity introduced into a test tube, diluted with isotonic salt solution to make a total volume of 9 c.c. and mixed. One-half c.c. of defibrinated blood was then added, and the whole thoroughly shaken. Control trials without these preparations were always started at the same time. The agglutination is readily detected in the foam or in a thin layer of the mixture. The observations extended over at least two hours, although the characteristic effects were apparent in the positive cases almost instantly with the larger doses, or within a few minutes at most. The microscopic appearance of the agglutinated corpuscles has been described by Müller.[1]

Müller has noted that the agglutinating action of commercial ricin preparations is evident in very dilute solutions. For defibrinated rabbit's blood (employed in a dilution of 1 volume of blood in 20 volumes) the limit of activity was reached with 0.01 per cent of ricin, quantities as small as 0.007 per cent being ineffective even after twenty-four hours' contact.

The results of many observations on the agglutinating power of our castor bean preparations are indicated in a few selected data, summarized from the protocols. No attempt is made here to indicate the rate of action in the individual trials or to determine accurately the exact limits of activity.

The table on page 281 shows the absence of agglutination in the trials with the globulin and proteose fractions. Bearing in mind that 1 mgm. of the product used represents, under the conditions of dilution selected, a solution of 0.01 per cent, it is evident that the limits of action for our ricin fall considerably below the figures set by Müller, and approach 0.001 per cent. The differences in the sensitiveness of different kinds of blood to the same doses of ricin may be due in part to the unlike proportion of erythrocytes contained therein. We have observed, for example, that quantities of ricin which failed to sediment 0.5 c.c. of blood might still agglutinate smaller quantities. Again, when larger doses of ricin were present, the clear supernatant serum would sediment additional quantities of corpuscles, whereas the fluid from trials with smaller doses failed to do this. These facts indicate a quantitative relation between the corpuscles and the ricin. Jacoby[2] has demonstrated that ricin is bound by the fixed

[1] MÜLLER: *Loc. cit.*, p. 311.

[2] JACOBY: Beiträge zur chemischen Physiologie, 1904, vi, p. 118.

receptors of the red blood corpuscles. Doubtless other factors also participate in determining the varying susceptibility of the blood from different species, thus explaining the relatively large doses of

SUMMARY OF TYPICAL SEDIMENTATION TRIALS.

Preparation used.	Kind of blood added.	Quantity of protein present in 10 c.c.	Agglutination and sedimentation, positive (+) or negative (−).
		mgm.	
No. 13. Albumin 70%, proteose 30%	Dog's	1.2	+ at once.
		0.4	+ slow
		0.23	+ slow
		0.2	+ slow
		0.04	−
	Pig's	0.3	+ at once.
		0.075	?
		0.045	−
	Cat's	1.0	+
		0.5	+
No. 15. Albumin 46%, proteose 54%	Dog's	0.55	+ at once.
		0.275	+ slow.
		0.16	?
		0.08	−
	Calf's	2.0	+ at once.
		0.6	?
	Sheep's	2.0	+
		1.0	?
	Cat's	1.0	+ at once.
	Hen's	4.0	+
		1.0	?
No. 12. Globulin and albuminate . .	Dog's	?	+
No. 16. Proteose	Dog's	11.0	−
No. 17. Proteose	Dog's	17.0	−
No. 18. Proteose	Dog's	12.1	−
No. 19. Proteose	Dog's	8.0	−
No. 10. Albumin	Pig's	0.1	+
		0.05	+
		0.03	?
No. 11. Proteose	Pig's	3.2	−
No. 4. Globulin	Pig's	7.0	−
Excelsin (globulin) from Brazil nut .	Pig's	2.0	−

ricin required to agglutinate hen's blood in our experiments (with Preparation 15) and fishes' blood in the experiments of Lau[1] and Fraenkel.[2] The latter investigator has shown that the resistance of the blood of the barbel towards ricin is not due to a lack of "receptors" in the corpuscles, but rather, in part at least, to the presence of a strong ricin-antiagglutinine in the blood serum of this fish. No antitoxic action is created by it, however.

The agglutinating property of our ricin is not thermolabile in the sense ordinarily applied to active sera; that is, it can be heated to 65° C. and higher for an hour without impairment of its characteristic sedimenting power. A dilute solution of Preparation 13, which did not show any incipient coagulation below 70° C., retained its agglutinating reaction when exposed to a temperature up to this point. We emphasize the fact that loss of this reaction is closely associated with coagulation of the albumin. No exact temperature can be stated, since this varies with the conditions under which the coagulation is attempted. The sedimentation was not observed to be noticeably hastened by keeping the tubes at 40° instead of at room temperature, 18°–20°.

The chemical nature of ricin. — The foregoing experiments leave little doubt, we think, that the physiologically active substance known as ricin is associated with the coagulable albumin of the castor bean. It remains to consider whether the toxine and agglutinine are identical with the protein, or merely present as contaminating compounds precipitated with it. The tendency of colloids to carry other substances, such as enzymes, with them when they are thrown out of solution is well recognized. Various considerations seem to us to speak against this explanation of the occurrence of ricin in conjunction with the castor bean proteins. Ricin is not obtained in the earliest (globulin) fractions with which impurities are usually deposited in fractional separations. The precipitation limits of the toxine and agglutinine are closely defined, falling between one-fifth and one-third of complete saturation with ammonium sulphate, and agree precisely with those of the albumin. Jacoby precipitated his ricin when the solutions contained 60 per cent of ammonium sulphate. Most pertinent, however, is the consideration of the extremely small doses necessary to give marked responses. If one-thousandth of a mgm. of a compound giving, on analysis, every indication of being a rela-

[1] LAU: *Loc. cit.* [2] FRAENKEL: *Loc. cit.*

tively pure protein, is physiologically active in the degree characterized by our experiments, the toxicity of any "impurity" must be infinitely greater than that of any known toxines.

The basis for the current statements regarding the non-protein nature of ricin has been derived from the experiments of Jacoby[1] in particular. In reviewing this work, Oppenheimer[2] wrote: "Es ist also wieder eines der letzten 'Toxalbumine' verschwunden, und damit dürfte wohl dieser Begriff auch nur noch historisches Interesse haben. Er hat in der Entwicklung dieser Frage seine grosse Bedeutung gehabt, besonders indem er zuerst darauf hinwies, dass diesen Giften ganz andere Eigenschaften zukommen, als den Krystalloiden Giften; aber nun dürfte es wohl an der Zeit sein, ihm ein ehrenvolles Begrabniss zu bereiten, da er jetzt nur noch Verwirrung stiften kann."

Turning to the actual experiment of Jacoby, we find that he digested the reprecipitated product derived from 400 mgm. of Merck's ricin with 100 c.c. of a trypsin solution for five weeks. Thereupon the fraction obtained by six-tenths saturation with ammonium sulphate was dissolved in 20 c.c. of 10 per cent sodium chloride solution, and its properties compared with an equivalent solution of the material before digestion. Protein reactions tried on the "purified" ricin were negative, but the toxic and agglutinating effects were maximal. In comparison with our own, Jacoby's "Ausgangsmaterial" must have been exceedingly impure; for it failed to dissolve in 10 per cent sodium chloride solution except as an "opaque solution from which a heavy precipitate at once separated on standing" (p. 38). Such an impure product may readily have undergone considerable digestion without loss of a small fraction of soluble toxic protein. Curiously enough, the "purified" ricin was quickly destroyed by trypsin. With reference to the negative biuret reaction obtained with 1 c.c. = 100 fatal doses per kilo of rabbit, it may be noted that our proteins were active in solutions far too dilute to give this test even after some concentration. (Cf. 13, p. 278.) In view of their importance, we have repeated the trypsin digestion trials upon one of our purer preparations. The digestive agents were Grübler's trypsinum purissimum and a very active pancreatic extract prepared from hashed pancreatic glands by extraction with toluol water, filtration, and long standing.[3] Protocols follow:

[1] JACOBY: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvi, p. 38.

[2] OPPENHEIMER: Toxine und Antitoxine, 1904, p. 164.

[3] MAYS: Zeitschrift für physiologische Chemie, 1903, xxxviii, p. 428.

I. *Dec. 23.* — 0.738 gm. of ricin, Preparation 15, was dissolved in 73 c.c. of the active filtered pancreatic extract, toluol added, and kept in a closed vessel at room temperature, later at 35°:

Solution A. 1 c.c. = 10 mgm. of the ricin preparation. 73 c.c. of the same pancreatic extract were preserved similarly with toluol for control trials = Solution B (no ricin).

Jan. 19. 1 c.c. of Solution A still gave a coagulum on heating, and agglutinated red corpuscles.

Feb. 7. $\frac{1}{10}$ c.c. of Solution A = 1 mgm. of the ricin preparation, failed to produce agglutination in dog's blood in the usual way; $\frac{1}{10}$ c.c. of Solution B + 1 mgm. of the ricin, Preparation 15, acted at once. $\frac{1}{2}$ c.c. of Solution A = 5 mgm. of the ricin preparation, still produced agglutination. A and B contained only traces of material precipitable by heat. With 1 c.c. of either the biuret reaction could not be demonstrated in the dark fluid.

May 21. 1 c.c. of Solution A = 10 *mgm. of the ricin preparation failed* to agglutinate cat's blood.

1 c.c. of the control solution B + 1 mgm. of the ricin, Preparation 15, was speedily active.

June 1. A rabbit of 2400 gm. received subcutaneously a portion of Solution A diluted and containing the equivalent of 0.72 mgm. of the ricin, Preparation 15, = 0.32 mgm. per kilo. This quantity of 15 represents several speedily fatal doses. The animal showed no toxic symptoms during the five weeks in which it was under observation.

II. *March 28.* 0.25 gm. of Preparation 15 was dissolved in 50 c.c. of the active pancreatic juice used in Experiment I, and put aside at 35° with toluol in a stoppered flask = Solution C. 1 c.c. = 5 mgm. of the ricin preparation.

For purposes of control another portion of the extract alone = Solution D, was also put aside.

May 21. 1 c.c. of Solution C = 5 *mgm. of the original preparation of ricin, failed* to sediment cat's blood; whereas 1 mgm. of the original ricin Preparation 15, added to 1 c.c. of Solution D (pancreatic extract), was active very speedily. $1\frac{1}{2}$ c.c. of Solution C showed no biuret reaction.

May 29. A rabbit weighing 1500 gm. received a subcutaneous injection of Solution C diluted, equivalent to 0.15 mgm. = 0.1 mgm. per kilo. Despite the fact that this dose had repeatedly been found to be fatal within thirty-six hours, the animal survived until June 3, when it was killed in a dying condition. The animal thus lived *five days*, indicating that the dosage of ricin was far smaller than calculated for the original preparation used. The autopsy findings were extremely typical.

III. *March 28.* 0.235 gm. of Preparation 15 + 0.5 gm. Grübler's trypsin were dissolved in 47 c.c. of water, toluol added, and placed in a stoppered

flask at 35°. The trypsin was tested with fibrin and found to be active. Solution E, 1 c.c. = 5 mgm. of the ricin preparation.

May 21. 1½ c.c. of Solution E still gave a faint pink-violet biuret reaction. 1 c.c. of Solution E = 5 mgm. ricin sedimented cat's blood speedily.

⅔ c.c. of Solution E = 2 *mgm. of ricin failed*, although 0.5 c.c. of the undigested ricin solution was effective on the same blood in comparable conditions.

May 29. A rabbit of 1500 gm. body weight received a dose of Solution E diluted, equivalent to 0.1 mgm. of the original ricin preparation. The animal was not perfectly healthy when used for the experiment, nevertheless it survived nearly four days instead of twenty-four to thirty-six hours.

EFFECT OF PANCREATIC DIGESTION ON RICIN.

	Period of digestion.	Agglutinating power.	Toxicity indicated in the rapidity of death with same dose.	
			Before digestion.	After digestion.
Experiment I.	4 weeks	Present	....	....
	6½ weeks	Diminished	....	....
	5 months	Lost	21 hours	Animal survived
Experiment II.	7 weeks	Lost	21 hours	5 days
Experiment III.	7 weeks	Diminished	21 hours	3 days

In all of these digestive trials both the toxicity and agglutinating power of the ricin has been impaired or destroyed along with the loss of protein properties. As was to be expected, if ricin is truly protein in nature, Solution E, in which the digestion had not advanced as far as in the other trials, retained more of its original activity. Any inhibitory action of substances derived from the digestive extracts was excluded by the control experiments with undigested ricin and comparable samples of the juices.

We have no evidence to offer at present regarding the probability of the independent existence of the active groups in the ricin molecule. Several writers have assumed that the toxic, agglutinative, and hæmorrhagic properties are distinct and separable. Müller found that Merck's ricin lost its agglutinating power after treatment with pepsin-hydrochloric acid, without disappearance of the toxicity of the solution. This fact, of itself, does not suffice to make the protein nature of ricin unlikely; for it is not difficult to conceive of a single

compound as having, in terms of Ehrlich's theory, both "toxophore" and "agglutinophore" groups, one of which is more readily destroyed than the other.

Summary.

The chemical study of the castor bean indicates that it contains proteins of the same character as the other oil-seeds which have been examined: namely, (1) a considerable quantity of a crystallizable globulin; (2) a much smaller amount of a coagulable albumin; and (3) proteoses. The elementary composition and reactions of these substances have been ascertained.

The physiological properties — marked toxicity and agglutination of blood corpuscles — ascribed to the substance known as ricin are associated with the coagulable *albumin* of the castor bean. This protein was isolated in a state of considerable purity without impairment of its solubility or physiological action, and an improved method for the separation of ricin is thus introduced.

Our ricin preparations retain a considerably higher toxic power than those heretofore described, a limit of 0.001 mgm. per kilo being exceeded as a fatal dose in rabbits. The toxic symptoms and the gross pathological conditions induced have been compared with those of previous investigators. The behavior of various species of animals towards the ricin albumin has been studied, and the susceptibility of frogs found to be greatly increased when they are subjected to a warm environment (above 25° C.).

The activity of the new preparations in agglutinating mammalian blood corpuscles is likewise very great, 0.001 per cent sufficing in some instances to sediment dilute blood suspensions. This power is not destroyed by heat below the coagulation temperature of the albumin.

The effect of trypsin on the active albumin (ricin) has been studied; the characteristic physiological properties are greatly impaired or destroyed by vigorous pancreatic digestion. A "purification" of ricin by such a method has failed.

The evidence respecting the protein nature of ricin has been discussed. The present investigation affords no satisfactory reason for denying the identity of ricin with the coagulable albumin of the seed.

THE MASS-MOVEMENTS OF THE CIRCULATION AS SHOWN BY A RECOIL CURVE.

By YANDELL HENDERSON.

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THE volume curve obtained by enclosing the heart in a plethysmograph connected with a tambour records the volume of each systolic discharge and the details of the filling and emptying of the pumping chambers. In the course of a series of observations on the volume curve of the heart in the dog, the writer was led to consider the need for a method of obtaining similar information as to the human circulation under normal and pathological conditions. To record the volume of the systolic discharge and the movements of the blood in the thoracic vessels of a normal man with the thoracic walls intact, would at first sight appear impossible. Yet at least three methods of attacking the various parts of this problem have been subjected to investigation.

The first of these methods to be studied with this end in view was that offered by the cardio-pneumatic curves. These curves express the movements of the air in the respiratory tract caused by the changes in the volume of blood in the thorax.[1] The utilization of these curves for clinical purposes has been attempted by Haycraft and Edie.[2] The difficulties of rendering the method practicable for clinical use proved, however, to be insurmountable.

A second possible line of attack on this problem is afforded by the X-ray shadow. The application of this method to the study of the movements of the alimentary canal has in the hands of Cannon proved extremely effective. The observation of the human thorax by this means has already been developed to a condition of great clinical importance. Yet an examination of the literature of the

[1] For a review of the extensive literature of this subject, see MELTZER, S. J.: This journal, 1898, i, p. 117.

[2] HAYCRAFT, J. B., and EDIE, R.: Journal of physiology, 1891, xii, p. 436.

subject and a few personal observations seemed to the writer to lend no encouragement for an attempt to obtain from this method data expressible in cubic centimetres of blood.

A third and very promising method of estimating the volume of blood discharged into the aorta at each heart beat has recently been described by Erlanger and Hooker. By the use of the sphygmomanometer in the improved form, devised by Erlanger,[1] the "pulse-pressure" (the pressure-height of the pulse wave, or the difference between systolic and diastolic pressure in the systemic arteries) is readily determined.[2] Erlanger and Hooker hold that (with certain limitations) the "pulse-pressure" varies directly with the volume of the systolic discharge of the heart.

The purpose of this paper is to describe still another method of estimating the volume of blood moved at each heart beat. While the results thus far accomplished fall far short of completing the investigation of the subject, they are sufficient to allow a brief preliminary report. These results are, however, not precisely those which were sought. The method here described gives not the volume changes of the ventricles, but a curve which expresses the algebraic sum of all the mass-movements of the circulation.

Probably every one has occasionally been kept awake by the rattling or creaking of his bed in unison with his heart beat. At such times one may notice slight but distinct vibrations of the entire body alternately headward and feetward. Consideration of the cause of these vibrations shows that the movement of the body in one direction must be due to a movement of blood in the opposite direction. If the body were supported in such a manner that its movements were not appreciably opposed by friction, it is evident that every mass-movement of blood headward or feetward would be associated with an exactly equal mass-movement of the body in the opposite direction. The principle involved is nothing more than that "every action has an equal and opposite reaction." It therefore during systole 100 gm. of blood were moved 7 cm. headward, the remainder of the body of a man weighing 70,100 gm. would be moved 0.01 cm. in the feetward direction. The centre of mass of the entire system (the body as a whole) would remain absolutely stationary. Furthermore, the

[1] ERLANGER: This journal, 1902, vi, p. xxii: and ERLANGER, J.: Johns Hopkins Hospital Reports, 1904, xii, p. 53.

[2] ERLANGER, J., and HOOKER, D. R.: Johns Hopkins Hospital Reports, 1904, xii, pp. 153–164 and p. 377.

arrest of the movement of the blood would bring the body to rest simultaneously. If there occurred a movement of blood headward, as in the veins, simultaneously with a movement of blood feetward, as in the aorta, the corresponding movement of the body would equal their algebraic sum. The curve obtained by recording these movements of the body by means of a lever tracing upon a moving surface affords, therefore, a direct quantitative expression of the algebraic sum of the mass-movements of the circulation. It is the Recoil Curve of the circulation as a whole.

So far as the writer has been able to learn, no observations on this subject are recorded in physiological literature, except a brief statement accompanied by a few tracings, which was presented by the writer before the American Physiological Society at its meeting in December, 1904.[1] The recoil curve is entirely different from the ordinary sphygmogram. It measures factors distinct from, and largely independent of, blood pressure. The "swinging table" described below is constructed on a different principle from the "balanced table" of Mosso.[2] The purposes to which the two pieces of apparatus are applied are wholly different.

The first records of the recoil curve were obtained by means of a plank suspended by wires from a high ceiling. The plank was free to move in every direction; and with a man lying upon it, had a pendulum period of about five seconds. A light lever magnifying the movements one hundred times was attached to the plank and arranged to write upon a smoked drum. At first a foot brace and handles were fastened on the plank, in order that the person under examination might attach himself firmly to the plank or "swinging table," as it may be called. The brace and handles were found to be unnecessary, however, as the weight of the subject is sufficient to carry the table through the full recoil movements, even when the person is fully dressed.

With such a table the heart beat causes not only longitudinal but also lateral movements. The latter have not yet been examined in detail. In fact it is necessary, in order to record the longitudinal movements with accuracy, that the lateral movements should be prevented. It is also necessary, not only that the person under examination should lie absolutely still, but that he stop breathing during

[1] HENDERSON: This journal, 1905, xiii, p. xxv.

[2] MOSSO, A.: Fear (English translation of fifth Italian edition by LOUGH, E., and KILSON, F.: 1896), chapter v, sections iii and iv.

the time of the heart beats recorded. Herein indeed lies the chief difficulty of the investigation, for while the total amplitude of the recoil movements is only a tenth of a millimetre, and some of the features of the curve amount to less than a tenth of this distance, respiration swings the body through a distance of many millimetres. Lastly, in order to avoid so far as possible errors from the table swinging back, after being moved out of plumb by the recoil, a pendulum period many times longer than the cardiac cycle was found necessary.

In attempting to avoid these difficulties several other forms of support for the table were tried. Thus the plank was placed on wheels on a track, and later on ball bearings such as are used for book-case doors. But this method proved to have even greater difficulties than the first. Next the plank was supported upon soft rubber stoppers. Upon each of six large stoppers a small stopper was placed. One pair was set under each corner of the plank and under the middle on each side. From the table so arranged tracings are readily obtained even while the person lying upon it is breathing normally. This great advantage is more than offset, however, by the elasticity of the rubber introducing a recoil of its own. Thus, while this method affords an easy demonstration of the existence of the recoil movements, it does not appear capable of yielding accurate curves. A similar difficulty was experienced in an attempt to record the tension of the recoil from the bending of a heavy spring made stationary at one end, and attached to the table at the other. Another method, which may be briefly mentioned, is the use of an ordinary balance. Upon the short arm of the balance the person was suspended in the standing or sitting posture, and the body weight counterbalanced by weights on the long arm. As this method practically doubles the mass to be moved, it was rejected together with the others above described.

On pages 291 and 292 is a brief description of the apparatus now in use.

As already stated, the greatest difficulty encountered has been to devise a method which would eliminate the swinging of the body and table under the influence of respiration. Various methods of damping have been tried. Thus it was found that if a small piece of cotton wool or felt be pressed lightly against the table, the respiratory movements practically disappear. The recoil movements of the circulation can then be recorded even while the subject is breathing. But with this arrangement the errors referred to in the discussion of

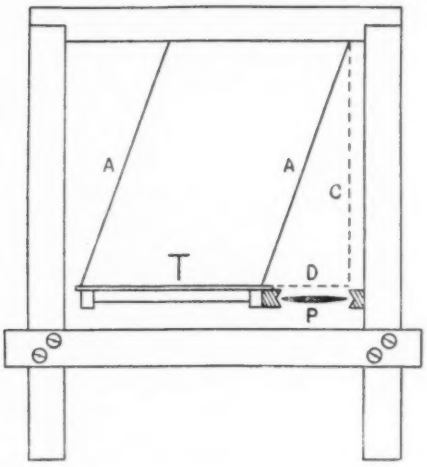

FIGURE 1. — A frame of heavy timbers was constructed somewhat like an old-fashioned four-poster bed. It is 2.0 metres long, 1.8 metres high, and 0.8 metre wide. One end is shown in the drawing. In this frame the swinging table *T* is suspended by four piano wires (*A*, *A*) fastened as shown, so that the motion is perpendicular to the plane of the drawing. The table itself is a thin plank 50 cm. wide and 1.2 cm. thick, supported and stiffened by stringers under each side connected by four cross pieces placed at equal intervals. It weighs 9 kilos. At each end of the table is a sharp-pointed steel pin *P*, 10 cm. long and 2 mm. thick. One end of each pin rests in a case-hardened steel cup sunk in the side of the table; the other end in a similar cup in the post of the frame. The latter pair of cups are adjustable in position both longitudinally and laterally. The use of these pins serves a double purpose. It limits the swinging table to longitudinal movements, the precise path of every point in the table being an arc of a circle whose radius is equal to the length of the pins. The second and more important object of the arrangement of the pins is to allow an adjustment of the table to a very slow pendulum swing. Thus if the dotted line *C* represent a plumb line dropped from the origin of the wire *A*, and *D* the horizontal distance from the insertion of *A* to *C*, it is evident that only when *D* is shorter than (or at most just equal to) the length of the pin, will the table rest in the position shown. As the steel cups in the frame are moved away from the table, its pendulum time is shortened; as they are moved out toward the table, the pendulum period is lengthened. Since the pins turn on their sharpened points with a minimum of friction, while the pendulum period can thus be lengthened almost to the vanishing point, the table not only moves under the influence of the slightest force (such as the flexing of a finger by a person lying upon it), but also remains stationary in the position at which it is brought to rest.

At each end of the table a stop is attached which by striking against the frame limits the total swing of the table to 8 mm. Fastened to the frame at one end is a clutch by which the table is held motionless while the person to be examined is lying down or rising and until the respiratory movements have ceased.

the arrangement of the table on rubber stoppers, are liable to appear. The method employed in obtaining the tracings herewith reproduced, was to provide the subject with a little whistle. On this whistle he was instructed to blow a long, steady, but not loud blast. The pressure developed within the thorax by blowing a small whistle is inconsiderable, while the arrest of respiration is sufficient for the purpose. It is difficult to get even an educated man to stop breathing for five or ten seconds. It is next to impossible to get the ordinary subject of treatment in a free clinic to do so. Persons affected with disorders of the circulation are furthermore especially short of breath. Asking the subject to blow on the whistle makes it possible, therefore, to obtain a recoil curve from persons whom it would otherwise be impossible to examine.

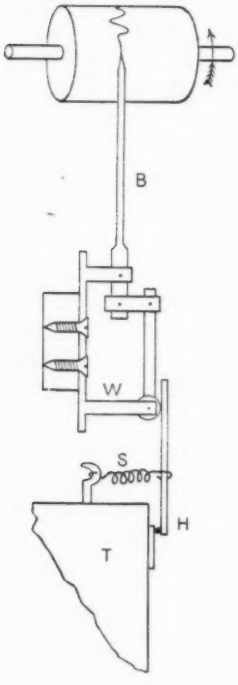

FIGURE 2.—In Figure 2 is shown the arrangement of the recording lever. Attached to one corner of the table *T* by the hinge *H* is a strip of stiff steel (15 by 1.2 by 0.2 cm.). By means of the spring *S* the strip of steel is held against the wheel *W*. This wheel is ground with the utmost precision to a diameter of 10 mm. Fastened to the wheel is an aluminum rod 10 cm. long which connects by means of a link with the lever *B*. The short arm of this lever, which is also of aluminum, is 3 cm. in length, the long arm to the writing point 15 cm. Thus every movement of the table is magnified 100 times with an error of probably less than 1 per cent. The advantages of the arrangement are that the connection between the table and lever is broken by simply loosening the spring, and that in any position of the table the lever is easily set to write upon any part of the smoked paper on the drum on which is recorded the recoil curve. Throughout these investigations a drum turning on a horizontal axis has been used, in order to avoid distortion of the recoil curve by the weight of the lever influencing the swing of the table.

In the tracings reproduced here, the pulse tracing was taken simultaneously with the recoil curve, by means of a small funnel pressed upon the throat over the carotid, and connected with a

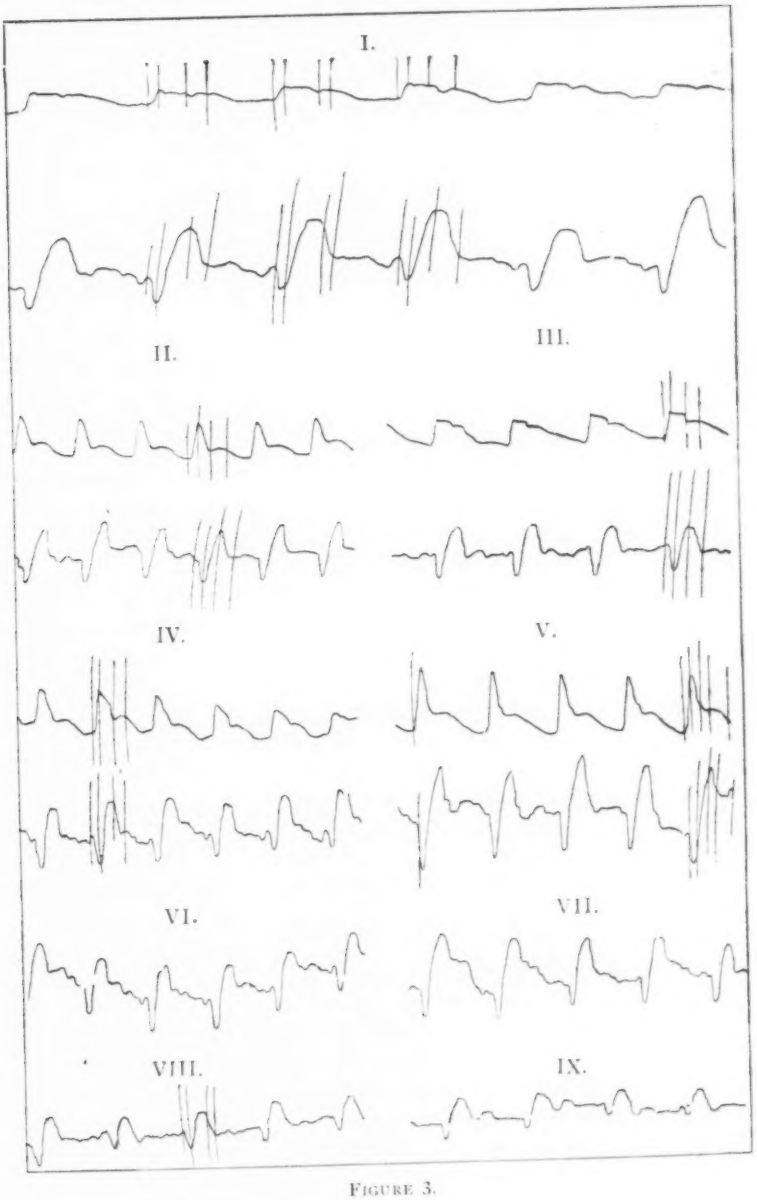

FIGURE 3.

tambour. The purpose of the sphygmogram is solely to indicate the positions in the cardiac cycle of the various features of the recoil curve. As the latter demands the undivided attention of the observer if accurate records are to be obtained, technical perfection in the pulse records has necessarily been sacrificed.

DATA IN CONNECTION WITH TRACINGS.

Tracing No.	Subject. Initials.	Age. Years.	Body weight.	Height.	Recoil movements.[1]			
					D_f μ.	D_h μ.	$D_f + D_h$ μ.	$\frac{D_f}{D_h}$.
			kilos	cm.				
I.	J. D. McG.	23	73	173	38	117	155	1 : 3.0
II.	R. H.	25	65	165	38	91	129	: 2.4
III.	H. D.	28	62	164	45	89	134	: 2.0
IV.	M. A. P.	20	71	173	44	107	151	: 2.4
V.	J. E. B.	25	70	180	68	158	226	: 2.3
VI.	F. M. A.	24	78	178	48	103	151	: 2.2
VII.	X.	20	61	171	44	123	167	: 2.8
VIII.	M. N.	26	65	163	28	61	89	: 2.2
IX.	H. E. S.	48	73	170	17	54	71	: 3.2

[1] The figures expressing the recoil distances, headward (D_h) and feetward (D_f) in micro-millimetres, are obtained by measuring the tracings, taking their average, dividing this by the magnification of the recording lever, and correcting for the weight of the swinging table by multiplying by the fraction $\frac{\text{weight of table + body weight}}{\text{body weight}}$.

In the analysis of the recoil curve, its various factors may be expressed by the equation $W \times D = w \times d$ or $\frac{w}{W} = \frac{D}{d}$; in which W is the body weight, D the distance through which the body is moved (or the length of the ordinates of the recoil curve), w the weight of the blood moved, and d the distance through which this blood is moved. How far the values of d are obtainable from anatomical data we shall not now stop to consider. For each point in the recoil curve this factor is probably some quite definite fraction of the height of the person examined. For purposes of comparison between persons of different heights it would be proportional to their height.

The factor d may therefore be replaced by h, the integer expressing the height. The equation then takes the form $\frac{D}{h} = \frac{w}{W}$. Now $\frac{w}{W}$ signifies the amount of blood per unit of body weight, moved in each cardiac cycle; while $\frac{D}{h}$ is the amplitude of the recoil curve corrected for the height of the person. For purposes of clinical comparison the amplitude of the recoil curve thus corrected affords therefore a measure of the volume of the systolic discharge of the heart per unit of body weight.

With this hypothesis the tracings, of which a few examples are herewith reproduced, are in complete accord. Thus, while the recoil curves from all normal persons exhibit the same features in the same relations to the sphygmogram, the scale of these curves varies greatly. Without exception the recoil curves of young men of athletic habits and more than ordinary muscular development are of considerable size (see tracings I and V). Tracings from women, and from men of sedentary habits and slight muscular development (especially when this condition is associated with a considerable amount of adipose tissue), are, on the contrary, of notably small size (as illustrated by tracings VIII and IX).

A further experimental test of this hypothesis is afforded by the observation that the recoil curve frequently exhibits a gradual diminution in amplitude with successive heart beats (as in tracing VII). This diminution almost invariably occurs when the subject blows too vigorously upon the whistle. It seems reasonable to infer that the increased pressure within the thorax thus produced, by opposing the flow of blood to the right auricle and venæ cavæ (as in Valsalva's experiment), diminishes the volume of blood which the ventricles discharge at each beat, — hence the diminution in the recoil of the body.

Figure 3 presents a summary of several hundred observations. In the first (A) of the three heart beats here represented, the horizontal parallel lines are drawn at distances equivalent to ten microns. Thus the curve is graduated to express the values of the factor D for normal men between twenty and thirty years of age, as calculated for a man of 170 cm. height upon a weightless table. In heart beat (B) are shown the directions of the recoil movements of the body with respect to the centre of gravity of the system, represented by the abscissa, together with the movements in the opposite direction of the blood represented by the dotted line. In (C) the relations of

the recoil curve to the carotid pulse are shown by synchronous lines.

Examination of this diagram reveals the following points:

(1) Shortly before the appearance of the pulse wave in the carotid, there occurs (D_i) a slight headward movement (1 to 4 microns). The cause of this movement, coming as it does before the discharge into the aorta and pulmonary artery begins, is probably to be found in the feetward movement of the blood in the heart and of the heart itself incident to the onset of systole.

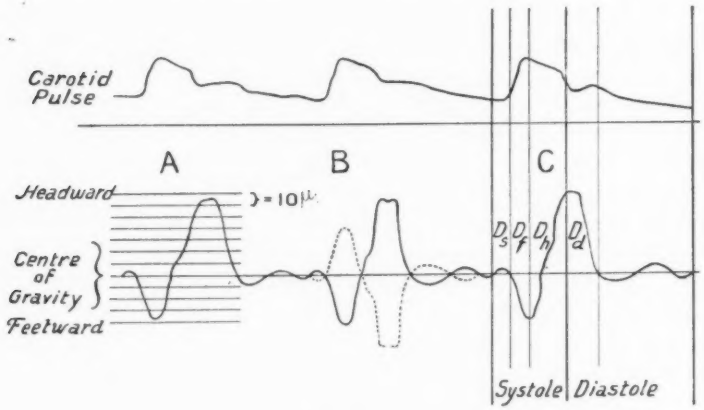

FIGURE 4.

(2) This slight movement is followed (D_f) by a rapid and considerable feetward movement (35 to 40 microns). As this jump of the body corresponds almost absolutely to the rise of the pulse wave, it seems beyond question to be assignable to the rush of the blood headward into the arch of the aorta, and into the large pulmonary arteries.

(3) The instant the pulse wave passes the arch of the aorta and starts feetward along this vessel, the body is thrown headward ($D_h = 90$ to 125 microns).

In respect to this movement, attention may be confined almost wholly to the events occurring in the left ventricle and in the aorta throughout its entire length; for it does not seem likely that the pulmonary circulation plays any considerable part in this headward jump. The reason for this opinion may be gathered by considering the anatomical relations as shown in Fig. 4. It can here be seen

that once the larger pulmonary arteries are distended, every cubic centimetre of blood, which is thereafter discharged from the right ventricle headward, will push an equal volume of blood feetward into the terminal arteries and capillaries of the lungs. Since these arterioles and capillaries are at approximately the same level as the ventricle itself, the recoils incident to the flow in opposite directions will largely neutralize each other. In the descending aorta, on the other hand, the distance through which the blood is moved feetward is relatively large. Thus the resultant of the discharge headward out of the ventricle, and of the movement feetward of the column of blood in the aorta, may be represented by the long arrow in Fig. 4. If, in order to illustrate this point, the length of the arrow be assumed to be 25 cm. (factor *d*), when the observed recoil of the body (factor *D*) is 125 microns, the weight of the blood moved must be 0.5 gm. per kilo body weight. In this reaction the flow of the blood in the carotid and brachial arteries will, of course, augment or diminish the extent of the recoil, according as the direction of its movement be feetward or headward. To some minor movement of this sort is perhaps to be assigned a minute notch which not infrequently occurs in the systolic rise of the recoil curve.

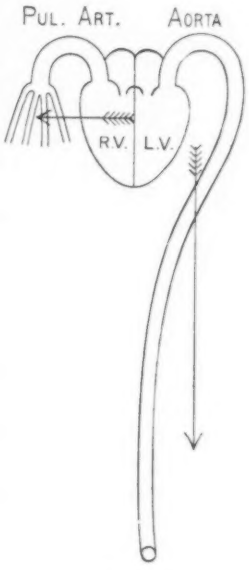

FIGURE 5.

In this connection it may be pointed out that, if the above explanations are correct, an exaggeration of the headward systolic recoil and a diminution in the preceding feetward recoil, should appear when the elasticity of the aorta is below normal, while the reverse should occur when there is an aneurism of the aortic arch. The discussion of these and related topics is left for a later paper.

(4) As the systolic discharge comes to an end and the dichrotic notch occurs in the carotid pulse curve, the headward movement of the body ceases. Then as the pulse rises in the secondary wave the body is moved feetward. In considering the cause of this first diastolic movement (D_d) two points are to be borne in mind, — that the

extent and direction of the recoil of the body indicates a considerable mass-movement of blood headward, and that the time occupied by this recoil corresponds with great regularity to the rise of the pulse curve from the bottom of the dichrotic notch to the summit of the secondary wave. Such a movement of blood at this time might be explained as due to a reflected wave returning headward along the aorta. But it seems to the writer much more probable that its correct interpretation is to be found in a rapid ebb of blood from the rear of the primary wave through the trough which follows this wave (represented in the sphygmogram by the dichrotic notch), into the arch of the aorta, where the blood thus collected forms the secondary pulse wave. The extent of the body's recoil to this diastolic movement appears to vary widely in different individuals.

(5) During the remainder of diastole, the only feature of the recoil curve which calls for notice is a slight wave which frequently occurs near the middle of the period.

In the foregoing discussion no reference has been made to the flow of the blood in the veins, because the slow and nearly uniform movement in these vessels probably plays little if any part in the recoil movements of the body. So also the filling of the ventricles during diastole makes no considerable mark on the recoil curve. This may be explained by supposing that while the volume of blood which flows from the auricles into the ventricles is large, the distance through which the blood thereby moves feetward is small. If these suppositions are correct, they lend further support to the view that the principal causes of the recoil movements of the body are to be found in the extensive movements of the blood incident to the pulse in the aorta.

Summary.

In this paper it is shown that under the influence of the mass-movements of the circulation, the body recoils at each heart beat feetward, headward, and again feetward. By means of a "swinging table" these movements can be magnified one hundred times and recorded in the form of a "recoil curve." The amplitude of these recoil movements of the body is held to be proportional to the volume of the systolic discharge of the heart.

In closing this paper the writer wishes to express his thanks to Dr. L. P. Wheeler, Instructor in Physics in this University, for valuable criticism.

SOME OBSERVATIONS ON THE ENZYME CATALASE.

By PHILIP SHAFFER.

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SCHÖNBEIN[1] was responsible for the belief generally held until a few years ago, that the decomposition of hydrogen peroxide was a property characteristic of all ferments. In 1892 Jacobson[2] reported some experiments which should have clearly proven the fallacy of this idea, but neither this author nor any following him have drawn such a conclusion from his work. Jacobson showed that there is no parallel between the catalytic action of preparations of the enzymes emulsin, trypsin, and diastase on hydrogen peroxide, and their other activities, and was able to separate the property of decomposing hydrogen peroxide from the other particular properties of these enzymes in several ways. Spitzer[3] later claimed that this catalytic property is not common to all ferments, but is due to the same enzyme which causes the blueing of guaiac in the presence of hydrogen peroxide, that is, the peroxidase.

Loew,[4] in 1901, showed this to be erroneous, and proved that the decomposition or catalysis of hydrogen peroxide is caused by a particular enzyme of very wide occurrence in the plant and animal kingdoms, which he called "catalase." This investigator isolated from tobacco leaves and from various other sources two varieties of this enzyme, α- and β-catalase. According to Loew the former is soluble only in dilute alkalis, which slowly decompose it with the formation of β-catalase. The β variety is soluble in water, and has the properties of an albumose. α-catalase he considers a nucleoproteid.

[1] SCHÖNBEIN: Journal für praktische Chemie, 1863, lxxxix, p. 323.
[2] JACOBSON: Zeitschrift für physiologische Chemie, 1892, xvi, p. 340.
[3] SPITZER: Archiv für die gesammte Physiologie, 1897, lxvii, p. 615.
[4] LOEW: Report No. 68, United States Department of Agriculture.

Is Catalase an Oxidizing Enzyme?

By isolating this enzyme as far as possible in a pure condition, Loew attempted to determine whether catalase is concerned in any reaction other than the decomposition of hydrogen peroxide. He decided from his experiments that, apart from this reaction, catalase is a true oxidizing enzyme. He pointed out that "the fact that catalase energetically decomposes hydrogen peroxide would not in itself justify the inference that catalase is also an oxidizing enzyme," but thought that such a deduction is probable on account of the analogous properties of platinum black. He based his conclusion, however, on the observation that supposedly pure preparations of β-catalase, obtained from the juice of poppy seed, sweated tobacco, etc., caused an oxidation of hydrochinone, giving after fifteen minutes "the characteristic quinone odor which after twenty-four hours became very strong." He tested for "oxidase" and "peroxidase" and for other enzymes in these preparations with negative results. He did not find that his preparations of catalase produced any other oxidizing reactions, and explained this on the ground of the specificity of enzymes.

It seems to me practically certain that the preparations used by Loew did contain, in addition to the enzyme catalase, other different enzymes or substances which produce the darkening of solutions of hydrochinone and its oxidation to quinone. In some experiments which I have carried out in conjunction with Dr. B. H. Buxton[1] on the enzymes in tumors, and in embryonic and normal tissues, no fresh animal tissues have been examined which did not give a reaction with hydrogen peroxide, indicating the constant presence of catalase, but frequently no reaction with hydrochinone was obtained. This fact would seem to be conclusive evidence against the statements advanced by Loew to prove that catalase is an oxidizing enzyme.[2]

Catalase is, however, usually classed with and treated in connection

[1] Buxton and Shaffer: Journal of medical research, 1905, viii, p. 543.

[2] The experiments mentioned above were carried on only with animal tissues. It seems not unlikely that the catalases of animal and vegetable origin may be quite different. Liebermann (Archiv für die gesammte Physiologie, 1904, civ, p. 204) found the catalytic power of animal tissue extracts much more resistant to heat than was the catalytic power of extracts of vegetable tissues. If the catalases are not identical, it may be possible that vegetable catalases have the property of oxidizing hydrochinone, though it does not seem probable.

with oxidizing enzymes, — oxidases and peroxidases.[1] Another reason for such a classification is doubtless the particular reaction which catalase brings about, — the decomposition of hydrogen peroxide with the evolution of oxygen.

Until very recently, so far as I am aware, no experiments have been attempted to determine whether in this decomposition the oxygen liberated is or is not in an atomic or *nascent* condition. In the decomposition of hydrogen peroxide by catalase as well as by colloidal metals, the final products are water and molecular oxygen.[2] If the reaction take place in some such manner as the following,

$$H_2O_2 \rightarrow H_2O + O,$$

whereby one atom of oxygen is set free from each molecule of peroxide, this nascent oxygen would be able to produce vigorous oxidations, and under such conditions catalase might properly be called an oxidizing enzyme. The reaction, however, does not appear to proceed in this way.

Recently Liebermann,[3] in a series of papers having as his purpose to learn the limits of analogy between organic and inorganic ferments, reported that in solutions of colloidal platinum which vigorously decomposed hydrogen peroxide he was able to demonstrate active or atomic oxygen. He was not able to detect active oxygen in extracts of malt and of other vegetable and animal tissues which likewise decomposed hydrogen peroxide vigorously. From a number of experiments, which it is not necessary to cite here in detail, Liebermann concluded that active solutions of colloidal platinum contain active oxygen, and furthermore that in the case of such "inorganic" ferments the reaction with hydrogen peroxide takes place as follows:

$$\text{Platinum-oxygen compound} + H_2O_2 \rightarrow \text{Platinum} + H_2O + O_2,$$

a part of the molecular oxygen again combining with two parts (molecules?) of platinum to give platinum-oxygen compound,

$$2Pt + O \rightarrow 2PtO.$$

[1] JACOBY: Ergebnisse der Physiologie, 1903, i, p. 213; OPPENHEIMER: Die Fermente und ihre Wirkungen, Leipzig, 1903; BACH and CHODAT: Biochemisches Centralblatt, 1903, i, p. 569.

[2] In his book, "Die Fermente und ihre Wirkungen," 1903, OPPENHEIMER twice states that hydrogen peroxide is decomposed into hydrogen and oxygen (pp. 350 and 352). This is of course only an oversight.

[3] LIEBERMANN: Archiv für die gesammte Physiologie, 1904, civ, p. 119.

From the fact that he was not able to detect active oxygen in extracts of plant and animal tissues, one is inclined to doubt, as did Liebermann, whether the mechanism of the reactions is the same in the two cases. He accordingly suggested a different explanation for the catalysis of hydrogen peroxide by extracts containing catalase. According to Liebermann the reaction in such cases takes place with a temporary oxidation of the enzyme by the peroxide with the formation of a true oxygen-enzyme compound, which reacts with another molecule of the peroxide with the liberation of *molecular* oxygen.

$$\text{ferment} + H_2O_2 \rightarrow \text{ferment} - O + H_2O$$

and

$$\text{ferment} - O + H_2O_2 \rightarrow \text{ferment} + H_2O + O_2.$$

This explanation seems at first very similar to his explanation of the catalysis of hydrogen peroxide by colloidal platinum. A fundamental difference is, however, that the platinum-oxygen compound is formed by the action of molecular oxygen, and is unstable, its oxygen being in a very active condition ; while the ferment-oxygen compound is formed only by hydrogen peroxide, or perhaps by similar organic peroxides, is comparatively stable, and its oxygen is not therefore in an active condition, being given up only to hydrogen peroxide with the formation of water and molecular oxygen, or to very easily oxidizable substances.

In the course of some experiments, planned originally for another purpose, I observed a fact which leads me to agree with Liebermann that active or nascent oxygen is not liberated in the action of extracts of animal tissues on hydrogen peroxide. When hydrogen peroxide is added to a solution of uric acid, the latter is slowly oxidized without any evolution of oxygen gas being observed. If enough peroxide is added, the uric acid after a few days is found to be entirely destroyed. If, however, there is a considerable concentration of the enzyme catalase present, the uric acid is *protected from oxidation*. This, I think, can be explained only by supposing that the oxygen liberated by the action of catalase on hydrogen peroxide is in a less active (molecular) condition than is the oxygen formed by the spontaneous decomposition of the peroxide (atomic oxygen). In accordance with this idea, there seems to be no reason for longer considering catalase as an *oxidizing* enzyme, for it not only reduces hydrogen peroxide, but appears to effect this in such a manner as to prevent

oxidations which would readily take place from the spontaneous decomposition of the peroxide. This protecting action of catalase seems to have some bearing on the question of the physiological function of the enzyme.

The Physiological Rôle of Catalase.

Loew[1] considers it very probable that hydrogen peroxide is formed in the tissues of the living organism by the processes of respiration and oxidation. There is but little satisfactory experimental evidence either in favor of or against this idea.

Bach and Chodat[2] have been able to show that peroxides are formed in certain vegetable cells, and these authors, as well as Kastle and Loevenhart,[3] think that the enzyme oxidase is of the nature of a peroxide. Bach and Chodat claim that the reaction of plant oxidases in blueing guaiac, liberating iodine from hydriodic acid, etc., is due to an activation of the peroxidase which is also present, by the oxidase, which acts just as does hydrogen peroxide. There is, in addition, the teleological argument that either hydrogen peroxide or a similar peroxide is very likely formed, since we find very widely distributed an enzyme which seems to have no other property than the decomposition of hydrogen peroxide. Until we have good reason to believe the contrary we may, perhaps, be justified in assuming that either hydrogen peroxide or a similar substance is formed in the body during the complicated processes of oxidation.

Accepting this premise, Loew assigns to catalase the rôle of destroying every trace of this substance (peroxide) as quickly as it is formed. In support of this view he mentions the strongly toxic action of hydrogen peroxide, and concludes that if it were not removed as fast as formed injurious oxidation of the protoplasm would result, ending in the death of the organism. His reasoning here does not seem very clear. Since the enzyme catalase is of such general distribution, the toxic action of H_2O_2 on the living organism must in all cases be, at least to a great extent, the result of its decomposition by the catalase. If it were possible to remove catalase entirely from a living cell, then it would be possible to determine the action of hydro-

[1] Loew: *Loc. cit.*, p. 42.

[2] Bach and Chodat: Berichte der chemischen Gesellschaft, 1902, xxxv, pp. 1275, 2465, 3943, and xxxvi, p. 606; Biochemisches Centralblatt, 1903, i, p. 417.

[3] Kastle and Loevenhart: American chemical journal, 1902, xxvi, p. 545.

gen peroxide itself on the protoplasm: but this cannot be done without the death of the cell. If it could be done, however, it is more than probable that some injurious oxidation would take place, for the experiments to be mentioned show one oxidation which proceeds readily by hydrogen peroxide acting alone but which is retarded or prevented if catalase is present.

Without some explanation, such as that proposed by Liebermann [1] for the mechanism of the reaction of catalase and hydrogen peroxide, it is difficult to understand the rôle which Loew has proposed for catalase. We know that hydrogen peroxide is a fairly strong oxidizing agent. Being quite unstable, it slowly breaks down with the formation of atomic oxygen, which is in a condition to produce very difficult oxidations.[2]

If the enzyme catalase is a true catalytic agent, as Loew seems to have believed it to be, it merely accelerates the natural slow decomposition of hydrogen peroxide, liberating in a given time a larger quantity of "active" oxygen, and so hastening any oxidation reaction which takes place more slowly under other circumstances.

With the very gradual formation of the peroxide, which has been assumed to take place in the organism, the catalase would of course prevent its accumulation, which would occur if the enzyme were not present, provided that the speed of formation of peroxide exceeded the speed of its spontaneous decomposition. But if the oxygen liberated from the peroxide by catalase were "active," the enzyme could hardly be considered as affording protection to the organism, for, as just pointed out, the active oxygen would be in a condition to bring about deep-seated oxidations, and the final result would be the same, whether the oxygen were liberated from the peroxide as fast as the latter is formed, or whether the peroxide were allowed to accumulate and its oxygen to be set free more slowly by spontaneous decomposition. If the speed of the spontaneous decomposition of the peroxide were greater than the speed of its formation, there would be no occasion for any catalytic agent.

[1] *Loc. cit.*, p. 193. LIEBERMANN does not, in the papers cited, consider this application of his explanation of the reaction. Indeed, he apparently considers it questionable as to what enzyme the decomposition of hydrogen peroxide is due, and does not discuss the subject.

[2] That atomic, or at any rate "active," oxygen is always present in solutions of hydrogen peroxide is shown by the very quick and strong reaction with potassium iodide-starch mixtures, and by other means.

In other words, if the reaction of catalase on hydrogen peroxide took place so that atomic or active oxygen were liberated, there would be, according to our present knowledge, no reason for supposing that the organism is protected by catalase from the toxic action of the peroxide, for the function of the enzyme would be merely to accelerate the strongly oxidizing action of atomic oxygen.

The function which Loew has assigned to catalase becomes clear, however, when we accept such explanation of the reaction as that suggested by Liebermann (see p. 302). According to this idea, the oxygen liberated in the reaction is in a molecular condition. My own experiments bear out this conclusion. Therefore, accepting the hypothesis that either hydrogen peroxide or a similar peroxide is formed in the organism, the enzyme catalase does protect the tissues by transforming the injurious, active oxygen of the peroxide into harmless and useful molecular oxygen.

Recently Vandevelde and Lebouq[1] have quoted Loew as stating that the function of catalase is the decomposition of hydrogen peroxide, with the liberation of atomic oxygen, which causes the strong oxidations which take place in the animal body. Vandevelde and Lebouq agree strongly with this view. Liebermann's experiments, as well as my own, together with the arguments stated above, seem sufficient evidence of the error of this idea.

Experimental.

The following experiments show, I think, that the enzyme catalase protects uric acid from oxidation by hydrogen peroxide, and so support, if they do not prove, the point advanced in the preceding pages, that the reaction proceeds in such a manner as to allow only the liberation of *molecular* oxygen.

Experiment 1. — A fresh beef liver was ground in a meat-chopper, and part of this liver hash, divided into four portions of 145 gm. each, was used for the experiment. Uric acid dissolved in lithium carbonate solution, the solution being then very nearly neutralized, was added to each portion of liver hash, the same amount of uric acid to each. The four portions were kept in stoppered bottles for seven days in an incubator at 38° C. Portions C and D were sterilized by heat to kill the catalase and other

[1] Vandevelde and Lebouq: Annales de la société de médecine de Gand, 1903, lxxxii, p. 237; Maly's Jahresbericht über die Fortschritte der Thier-Chemie, 1904, xxxiii, p. 1080.

enzymes before being placed in the incubator. To B and D hydrogen peroxide was added slowly and with stirring, 30 c.c. each day (200 c.c. 3 per cent solution to each during seven days).

At the close of the digestion, the content of each bottle was made up to about 900 gm., heated to boiling in a steam sterilizer for one hour, and, after cooling somewhat, was made up to exactly 1000 gm. and filtered.

The uric acid and xanthin bases were determined in portions of the filtrate in this experiment and in those following, by a modification[1] of the Ludwig-Salkowski method for uric acid, and by the Salkowski method[2] for xanthin bases.

Uric acid and xanthin bases were precipitated together with the phosphates by magnesia mixture and ammoniacal silver nitrate. After filtering and washing, the precipitate was decomposed with hydrogen sulphide in hydrochloric acid solution, boiled, filtered, and the clear filtrate evaporated on the water bath to about 30 c.c. After standing over-night, the uric acid crystallized out was filtered, washed, dissolved in a little sodic hydrate solution, and titrated, after the addition of 15 c.c. concentrated sulphuric acid, with $\frac{n}{20}$ potassic permanganate (1 c.c = 3.75 mgm. uric acid). When there is much xanthin base present, there is danger of it separating out on standing, thus contaminating the uric acid. When this occurs, it is readily detected by the appearance of the precipitate, which is scaly, or very finely divided, and amorphous, causing the mother liquid to be turbid or milky. In such cases the liquid was filtered and the precipitate dissolved in a little dilute sodic hydrate, and the volume made up to about 200 c.c. Then 20 c.c. of 10 per cent sulphuric acid was added, and the liquid again evaporated on the water bath to about 40 or 50 c.c. On standing, the uric acid separated fairly pure.[3] The xanthin bases were precipitated in the combined filtrates and wash-water from the uric acid, by ammoniacal silver nitrate, the precipitate washed with ammonia water till the filtrate was free from silver, then dried and ignited in a porcelain crucible. After dissolving in nitric acid, the silver was titrated with $\frac{n}{10}$ ammonic sulphocyanate (1 c.c. = 1.52 mgm. xanthin), the whole of the xanthin bases being calculated as xanthin.

Phosphates were determined in the filtrates from the autolysis mixtures by precipitation with magnesia mixture, filtering after twenty-four hours, and titrating the precipitate, dissolved in acetic acid, with uranium acetate.

[1] FOLIN and SHAFFER: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 552.

[2] SALKOWSKI: Archiv für die gesammte Physiologie, 1898, lxix, p. 280. NEUBAUER and VOGEL: Analyse des Harns, 1898, 10th edition, p. 829.

[3] HORBAZEWSKI: Zeitschrift für physiologische Chemie, 1894, xviii, p. 341.

The following results of Experiment 1 are calculated for the total 1000 gm. of each portion, from averages of duplicate determinations:

	Uric acid.	Xanthin.	P_2O_5.
	gram	gram	gram
A. Liver hash + uric acid	0.423	0.201	0.593
B. Liver hash + uric acid + H_2O_2 added each day	0.505	0.051	0.670
C. Liver hash + uric acid Sterilized before digestion	0.346	0.050	0.320
D. Liver hash + uric acid Sterilized before digestion + H_2O_2 added each day	....	....	0.358

It will be noted that in B more free phosphates were found than in A, probably indicating the breaking down of a larger quantity of nucleoproteid, which also gives the xanthin bases and, by further oxidation, uric acid. Hence, if no destruction of uric acid or nuclein bases has been produced by the hydrogen peroxide, we should expect a larger amount in B than A. The 0.201 gm. of xanthin in A is equivalent to 0.222 gm. uric acid; or A contained the total equivalence of 0.222 + 0.423 = 0.645 gm. uric acid. There was probably some slight destruction of uric acid, but it is quite inconsiderable when compared with the destruction in D, in which the catalase had been killed before the hydrogen peroxide was added. Here no uric acid was found.

Experiment 2. — In this experiment an emulsion of bacteria (Bacillus isolated from urine) was used to furnish the catalase. A twenty-four hour culture was made in six potato tubes of agar, and an emulsion made of the bacteria in 150 c.c. water. The emulsion decomposed hydrogen peroxide vigorously, but gave not the slightest reaction with guaiac and hydrogen peroxide for peroxidase.

A. 50 c.c. bacteria emulsion + 150 c.c. uric acid solution (neutralized immediately before adding to bacteria).

A served as a control for B and C. After three days at 38° C. it was heated to boiling, diluted to 500 gm., and filtered; the uric acid determinations[1] gave 0.453 gm. and 0.435 gm.

B. 50 c.c. bacteria emulsion + 150 c.c. uric acid solution (neutralized). Added 60 c.c. 3 per cent hydrogen peroxide solution each day for three

[1] In this experiment, there being no xanthin bases present, the uric acid was determined by precipitation with NH_42SO_4, etc., as proposed by FOLIN and SHAFFER: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 552.

days. Kept three days at 38° C. Made up to 500 gm., heated to boiling, filtered, and determined uric acid. Total = 0.448 gm.

To a portion of this liquid, in which the catalase had been killed, there was added one-fifth of the same amount of 3 per cent hydrogen peroxide. After standing two days at 38° C., some hydrogen peroxide was still present in the liquid. Uric acid was determined both before and after heating on the water bath, and the results calculated for the whole of the original 500 gm. liquid.

Before boiling, 0.103 gm. uric acid.
After boiling, 0.098 gm. uric acid.

Therefore there was no loss of uric acid when catalase was present, but when the catalase was killed the amount of uric acid destroyed was more than three-fourths of the whole, 0.340 gm.

C. 150 c.c. neutralized uric acid solution + 100 c.c. water. Added 60 c.c. 3 per cent hydrogen peroxide in three days, 20 c.c. each day. After three days at 38° C. uric acid was entirely destroyed.

D. 150 c.c. neutralized uric acid solution + 25 c.c. of a clear liver extract (catalase). Added 60 c.c. 3 per cent hydrogen peroxide each day with stirring, for three days. Recovered 0.454 gm. uric acid.

Experiment 3. — An extract was made from 3 kilos of hashed beef liver and 4500 c.c. salt solution. Two portions of this extract, 1000 c.c. each, were allowed to autolyze for three days at 40° C., during which time a current of air was passed through both. Chloroform and toluol were added to prevent bacterial growth. On the third day 300 c.c. water was slowly added to one, and 300 c.c. 3 per cent hydrogen peroxide to the other. The dropping tubes extended well below the surface of the liquids, and the mixtures were well stirred by the air current. The catalase was strongly active. After the water and hydrogen peroxide had run in, the autolysis was stopped, the liquids were made very slightly alkaline, heated on the water bath, acidified, heated again, made up to 1700 gm. each, and filtered from the coagulated proteid. In the filtrates the uric acid and xanthin bases were determined in duplicate. The results follow:

	Total uric acid.	Total xanthin.
	mgm.	
A. Liver extract + hydrogen peroxide	151	0
	135	0
B. Liver extract + water	91	0
	100	0

Experiment 4. — In order to learn whether catalase protects also xanthin from oxidation, the following experiment was carried out. Xanthin was dissolved in dilute potassium hydrate solution, and nearly neutralized immediately before use. 120 c.c. of this solution contained 70.8 mgm. of xanthin.

A. 120 c.c. slightly alkaline solution of xanthin + 30 c.c. liver extract (catalase) + 50 c.c. 3 per cent hydrogen peroxide added slowly with stirring. After three days determined xanthin. Found total 72.3 mgm. and 68.4 mgm.

B. 120 c.c. slightly alkaline solution of xanthin + 30 c.c. water + 50 c.c. 3 per cent hydrogen peroxide. After three days some hydrogen peroxide still present. Total xanthin found = 2.7 mgm. and 3.6 mgm.

The foregoing experiments show very clearly that catalase does in some way protect the uric acid and xanthin (and probably also many other substances) from oxidation by hydrogen peroxide. It seemed quite possible, however, that this might be entirely caused by the hydrogen peroxide being quickly decomposed at the surface of the liquid or in one place, and so prevented from coming in contact with the uric acid in the greater part of the solution. In order to test this point as far as seemed possible, the following experiment was carried out:

Experiment 5. — A. To 300 c.c. slightly alkaline solution of uric acid was added 100 c.c. of a dilute clear liver extract, with some chloroform to prevent putrefaction. After three days at 38° C. the liquid was made slightly acid, heated on the water bath to coagulate the coagulable proteid, made up to 600 gm., and filtered.

A serves as a control on the following.

The uric acid determinations gave a total of 0.477 gm.

B. 300 c.c. uric acid solution + 100 c.c. dilute liver extract (catalase). 100 c.c. 3 per cent hydrogen peroxide was added very slowly, drop by drop, from a dropping funnel which extended below the surface of the liquid. A turbine stirred the liquid vigorously while the peroxide was run in, so that the latter was decomposed slowly and uniformly throughout the whole liquid. After three days the liquid was made up to 600 gm., the albumen coagulated, and the uric acid determined in the filtrate as in A.

Total uric acid = 0.276 gm., or a loss of 0.201 gm.

C. Same as B, except that hydrogen peroxide was added at once and the liquid was not stirred.

Total uric acid = 0.272 gm., or a loss of 0.205 gm.

D. 300 c.c uric acid solution + 100 c.c. water. No catalase. 100 c.c. hydrogen peroxide added at once. After three days,

Total uric acid = 0.126 gm., or a loss of 0.351 gm.

In B and C of this experiment the concentration of the catalase was so low that the hydrogen peroxide became thoroughly mixed with the solutions before it was decomposed, and yet very much less uric acid was destroyed than in D, which contained no catalase. Moreover, in B and C all of the hydrogen peroxide added was decomposed during the three days, while in D much hydrogen peroxide was still present when the experiment was stopped. If the catalase had acted on the hydrogen peroxide in such a way as to set free the oxygen in an active condition, as it is in the spontaneous decomposition of hydrogen peroxide, more active oxygen would have been formed in B and C than in D, and we should have found a greater destruction in the two former than in the latter. That a considerable amount of uric acid was destroyed in B and C is to be explained, I think, by the slowness of the decomposition of the hydrogen peroxide by the catalase, and the consequent presence always in the liquid of hydrogen peroxide which was not taking part in the reaction with the enzyme and was free to attack the uric acid. If for any reason the catalase is wholly or very largely destroyed, the hydrogen peroxide readily oxidizes the uric acid. The continued addition of hydrogen peroxide, an acidity of the liquid above $\frac{n}{30}$ (HCl), or even driving air through the solution for a long time will greatly reduce or even destroy the activity of this enzyme. If hydrogen peroxide is added for a long time in large quantity, catalase is gradually destroyed, and only as this destruction proceeds is the oxygen of the peroxide available to the uric acid. The following experiments indicate this:

Experiment 6. — An autolysis of extracts of beef liver was continued for seven days at 38° C.

A. 500 c.c. liver extract. 500 c.c. water added in 25 c.c. portions in seven days. After coagulating and filtering, uric acid was determined in the filtrate.

Total uric acid = 0.251 gm.

B. 500 c.c. liver extract. Added 500 c.c. 3 per cent hydrogen peroxide in 25 c.c. portions during seven days. At the close of the autolysis the catalase was almost entirely gone. No uric acid was found.

Experiment 7. — A. 500 c.c. liver extract. Added 500 c.c. water, drop by drop, during seven days. An air current was run through the liquid from 9 A.M. to 6 P.M. each day for seven days.

In the filtrate from the coagulated albumen there was found a total of 0.304 gm. uric acid.

B. Duplicate of above, except that 500 c.c. hydrogen peroxide was added instead of water. The dropping tube extended nearly to the bottom of the flask, and the liquid was well stirred with the air current. At close of autolysis, catalase had been entirely destroyed.

Total uric acid found = 0.030 gm.

Experiment 8. — Two portions of beef liver hash, 150 gm. each, mixed with 1500 c.c. salt solution, were autolyzed for two weeks at 38° C. During the day a current of air was passed through the liquids, as in the preceding experiment. Chloroform and toluol were added daily.

A. Control. 2000 c.c. water was added slowly and constantly during two weeks. At the close of the autolysis the liquid was boiled, made up to a definite volume, and filtered from the coagulum. In the filtrate there was found

Total uric acid = 0.455 gm.
Total xanthin = 0.289 gm.

B. 2000 c.c. 3 per cent hydrogen peroxide was added constantly during two weeks. After ten days the catalase had entirely disappeared, and at the close of the autolysis some hydrogen peroxide was present in the liquid. In the filtrate was found

Total uric acid = 0
Total xanthin = 0.360 gm.

The bearing of these experiments on the questions regarding whether catalase should be considered an oxidizing enzyme and regarding the physiological function of catalase, has been discussed earlier in this paper. It remains to summarize very briefly the general conclusions.

Experiments are described which, it is believed, confirm the idea, advanced by Liebermann, that in the action of extracts of plant and animal tissues on hydrogen peroxide, in which the peroxide is decomposed with the evolution of oxygen gas, the oxygen liberated is only in a *molecular* condition, as distinguished from the active or atomic oxygen which is liberated in the spontaneous decomposition of hydrogen peroxide. This explanation of the reaction produced by catalase appears to have a bearing on the properties and function of the enzyme. The conclusions are reached that catalase is not an oxidizing enzyme; and, provided we accept the assumption of the formation of hydrogen peroxide or a similar peroxide in the organism, that the physiological function of catalase is to destroy the peroxide in such a manner that the oxygen is not liberated in an active condition,

and thus to protect the tissues from the injurious oxidation which the peroxide would otherwise effect.

Since writing the above, a paper has appeared from Leo and Paul Liebermann,[1] showing that catalase is not necessary for the guaiac reaction. Loew came to the same conclusion in his original paper on catalase (*loc. cit.*). Additional proof of the correctness of this idea is the fact that, if extracts of animal tissues containing both catalase and peroxidase — extract of liver, for instance — be acidified slightly (to $\frac{n}{20}$), and hydrogen peroxide then added, no evolution of oxygen takes place, although on the addition of guaiac solution the blue color immediately appears. The fact that catalase is not necessary for this reaction, which seems to be brought about by some form of active oxygen, might perhaps be accepted as an indirect argument in favor of the point forming the basis of this paper, — that very probably only inactive, molecular oxygen, and not active oxygen, is liberated during the reaction of catalase on hydrogen peroxide.

In connection with the discussions of this paper, the reader is referred to the work of Kastle and Loevenhart,[2] which unfortunately I did not see until my paper had been for some time in the hands of the printer. Kastle and Loevenhart concluded that in the catalysis of hydrogen peroxide by either catalase or finely divided platinum, the oxygen liberated is in a molecular condition. A fundamental distinction exists, however, between the action of the enzyme catalase and that of colloidal platinum, as has been pointed out above (see page 302).

[1] LIEBERMANN, L. and P.: Archiv für die gesammte Physiologie, 1905, cviii, p. 489.

[2] KASTLE and LOEVENHART: American chemical journal, 1903, xxix, p. 397.

EXPERIMENTAL STUDIES ON THE PHYSIOLOGY OF THE MOLLUSCS — SECOND PAPER.[1]

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The Inorganic Constituents of the Liver of Sycotypus.

IN the preceding paper we touched upon the liver of Sycotypus purely as a digestive gland, and without reference to its other functions. In this communication the liver will be considered more in detail and with especial reference to its minute structure, its stored metallic elements, its relation to the blood, and its characteristic chemical composition.

TABLE I.

Total weight of animal.	Weight of liver.	Proportion of liver weight to total weight.
grams	grams	per cent
121	11.2	9.3
152	17.5	11.6
193	20.4	10.6
199	21.5	10.8

The liver of Sycotypus canaliculatus, as that of all the spirally arranged gasteropods, is the large glandular organ occupying the summit whorls of the shell. Its size and mass indicate the importance of the organ in the economy of the anima'. In a number of average specimens the liver was found to comprise approximately one-tenth of the total body weight, including the shell. A series of typical observations are recorded in Table I.

[1] The first paper was published in this journal, 1905, xiii, p. 17.

The liver is always a highly pigmented organ, varying in different animals from a yellow brown to a dark green or blackish hue. The color depends upon two factors: the yellow liver secretion present in the cells and tubules, and the pigment cells of the inter-tubular connective tissue. The latter are very characteristic of this particular mollusc in respect to their chemical composition; as will be shown later, they are connected closely with the animal's capacity for storing metallic elements, and are thus intimately related to the metals present in the respiratory proteids of the blood.

It was pointed out in the first paper that the three large diverticula entering the liver from the stomach act as cœca in which the major part of the fats and carbohydrates is digested, and also as hepatic ducts through which the brown liver secretion is emptied into the stomach after digestion is completed, or nearly so. It seems most probable that this brown pigmented secretion is derived from the transformation of the blood proteid, in a way analogous to the formation of bile by the vertebrate hepatic cell from its antecedent hæmoglobin. The absence of bilirubin and biliverdin is to be expected, since the blood of Sycotypus contains no detectable amounts of hæmoglobin.

The presence or lack of bile pigments and bile salts was for many years a mooted question in the physiology of invertebrates, though recently the refinement of methods has made it possible to disprove entirely their occurrence among the molluscs at least. Earlier investigators claimed to find in the hepatic secretions of molluscs compounds which give Gmelin's reaction; but Krukenberg[1] reinvestigated this point, and failed to obtain a trace of bile pigments or bile salts in any of the animals examined. In the liver of Sycotypus no reactions have been obtained for either of these characteristic products of the vertebrate liver.

One of the most interesting functions of the molluscan liver, as typified by that of Sycotypus, is its power of retaining and storing up those elements which are necessary for the building and repair of the shell, and those which are characteristic of the circulating respiratory proteids. In his classic paper upon the molluscan liver Barfurth[2] pointed out the large calcium and magnesium content of the livers of various gasteropods, stored there evidently for the repair of the

[1] KRUKENBERG: Untersuchungen des physiologischen Instituts, Heidelberg, 1878, ii, p. 2

[2] BARFURTH: Archiv für mikroskopische Anatomie, 1882, xxii, p. 473.

shell, the secretion of the epiphragma, etc. The content of these elements is found to vary also with the season — a larger percentage is normally present in the liver before the secretion of the epiphragma than afterwards. Among others, Dhéré[1] showed the connection between the copper in the blood of those invertebrates having hæmocyanin, and the copper normally found in the livers of the same.

In the liver of Sycotypus calcium and magnesium are abundant; they are probably common to the livers of all shell-building molluscs and need not be discussed here. The two elements copper and zinc are, however, somewhat unique. Copper is found in all hæmocyanins as the characteristic metallic element, and is found stored in the livers of hæmocyanin-bearing animals. Zinc, on the other hand, — undoubtedly in a somewhat similar proteid combination, — is an element characteristic of this animal alone, as far as we have observed, and may even prove to be a somewhat local phenomenon peculiar to those specimens growing about Long Island Sound.

Zinc was first definitely identified and estimated in the ash of Sycotypus in 1903.[2] The results of analysis made at that time and several subsequent periods to discover whether its presence is a normal and constant phenomenon or not, are presented in the next table. They serve to show the fairly uniform amounts present in lots collected from widely separated portions of Long Island Sound at varying intervals of time. As is evident from the data, the three elements, iron, copper, and zinc, have never been missed in qualitative examinations extending over nearly two years, while the quantitative estimations show a nearly uniform amount of copper and zinc present. The assumption therefore that zinc as well as copper is a constant and normal constituent of the liver tissue of Sycotypus seems warranted.

Other tissues of Sycotypus were also ashed and analyzed; but with the exception of the blood, no other tissue was found to contain zinc. Various other marine animals, including some of the most common molluscs and crustacea of the coast were examined for zinc. With the exception of the common "drill," Urosalpinx cinerea, no appreciable amounts of the element could be found.

Before proceeding to discuss in detail this unique occurrence of zinc in the liver and blood of Sycotypus, it may be well to describe the anatomy and histology of this organ somewhat more fully than was attempted in the earlier paper. We have evidently in the liver

[1] Dhéré: Comptes rendus de la société de biologie, 1903, lv, p. 1912.

[2] Bradley: Science, 1903, xix, p. 196.

a type of gland representing relatively slight specialization; a gland performing the functions which in higher types are carried out by several highly differentiated organs. Its histology is therefore of interest in that it represents the structure of an organ highly developed and of primary importance to the animal, but not specialized in function.

TABLE II.

THE PRESENCE OF IRON, COPPER, AND ZINC IN THE LIVER ASH.

Date.	Iron.	Copper.	Zinc.
	per cent	per cent	per cent
May, 1903	present	8.57	9.62
May, 1903	"	8.17	8.69
September, 1903 . . .	"	8.47	15.27
November, 1903 . . .	"	7.83	18.79
November, 1903 . . .	0.84	present	15.11
November, 1903 . . .	0.84	"	14.94
May, 1904	present	9.41	15.41
June, 1904	"	9.37	14.22
September, 1904 . . .	"	present	present
October, 1904	"	"	"
November, 1904 . . .	"	"	"
May, 1905	"	"	"

The structure of the liver of Helix as studied by Barfurth[1] is in a general way characteristic of other gasteropods also. The gland is of the compound tubular type, the tubules discharging finally into the three diverticula or hepatic ducts. In the secreting tubules Barfurth distinguished three types of cells, assigning to each a definite function. Thus there are liver cells, filled with globules of secretion, brown in color and corresponding to the bile of vertebrates; ferment cells, filled with the digestive enzymes; and calcareous cells, of different size and shape, in which are to be found glistening particles of calcium phosphate, soluble in acids. It may be doubted, however, whether the chemical and other evidence proving the identity of these cells is very exact or conclusive. Although somewhat similar variations are

[1] BARFURTH: Archiv für microskopische Anatomie, 1882, xxii, p. 473.

found in the cells of Sycotypus, we have no evidence to show which cells are hepatic, which contain ferments, and which are merely storage cells for reserve material. Indeed the evidence concerning the latter function at least, tends to show that the cells of the secreting tubules are not themselves greatly differentiated, and that probably a single cell may perform all three functions mentioned. Two distinct types of cells appear in the sections: the long cylindrical epithelial cells of the tubules, filled with the secretory granules, and the broad, light staining cells, corresponding to the calcareous cells of Barfurth's figures. The number of these is very much smaller than of the former.

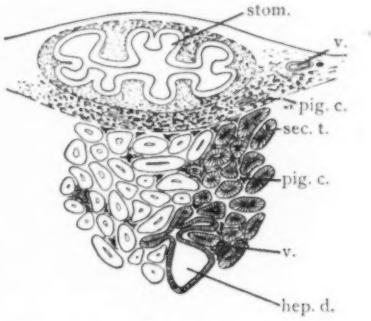

Diagram of a section of the liver and stomach near the pyloric end of the latter organ. Between the liver and stomach (*stom.*) is the sheath of vascular connective tissue with its blood vessels (*v.*), and pigment cells (*pig. c.*). The occasional blood vessels of the liver, the secreting tubules (*sec. t.*), the pigment cells of the interstitial connective tissue, and a section of one of the hepatic ducts (*hep. d.*), are indicated. See also Figure 8 in our first paper.

A delicate basement membrane and a network of interstitial connective tissue separate the tubules. This network forms a vascular space for the gradual movement of the blood stream throughout the organ; for although the hepatic vessels are found within the liver mass, they occur so rarely that the chief supply of blood must reach the secreting cells through these meshes of the vascular connective tissue. On the other hand, in the connective tissue about the stomach and intestine where absorption is going on, and in the capsule-like membrane surrounding the liver as a whole, blood vessels and their accompanying nerves are abundant.

Most characteristic of the liver of this gasteropod are the dark green pigment cells lying in the connective tissue between the tubules, especially prominent in the sheath about the stomach and surrounding the blood vessels. The diagram shows these cells and the general structure of the liver as seen in typical sections.

The ash. — In examining the liver of Sycotypus chemically, one is impressed by the readiness with which the dry tissue is reduced to a carbon-free ash, and the large amount of the latter. So easily is the

oxidation performed, that a section may be ashed on its slide with no appreciable curling of the tissue or displacement; so that the resulting ash represents very accurately the general distribution and comparative amounts of the inorganic salts. In preparing these ashed sections, thin glass slides were used, the microtome section mounted in the usual way, the paraffin removed by xylol, and the xylol by alcohol. By drying and gradually heating, such a section can be oxidized perfectly *in situ*, until finally by igniting to a red heat a carbon-free ash is left which adheres by incipient fusion to the glass and may then be used for microchemical tests or made permanent by covering with balsam. The ash was found to retain the form of the original tissues so perfectly that not infrequently the individual cells were outlined. The amount of ash in the intestinal mucosa is insignificant, while that of the liver cells proper and the interstitial connective tissue is large. By appropriate microchemical reactions it was possible to show that practically no copper is present in the ash of the intestinal mucosa, while the liver tissue itself is rich in both copper and zinc. The most satisfactory microchemical test for zinc in such cases was found to be its reaction with a soluble nitroprusside. If the section under cover glass was flowed with a drop of an acid solution of sodium nitroprusside and warmed, zinc nitroprusside formed and upon cooling was deposited in characteristic crystal form. These crystals, quite insoluble, can readily be distinguished, when examined under high power, from the amorphous particles of the copper nitroprusside which forms at the same time.

The important result of these microchemical reactions with the ash is to show the presence of sufficient copper and zinc to give definite tests in a microtome section. The possibility of devising tissue stains by which the copper and zinc may be recognized in the tissue itself is at once suggested.

Tissue stain for copper. — Several reactions for copper were found by which its presence in combination and its distribution in the tissues could be definitely shown. Of these the reactions with ammonium sulphide, potassium ferrocyanide, and dilute hæmatoxylin are permanent and quite delicate, while the well-known color reaction produced by a solution of hydrobromic acid in which there is a trace of free bromine is brilliant and very delicate, but transient. The color compound fades and rapidly diffuses throughout the tissue, so that the reaction must be observed and noted as it goes on. The first three reactions named were employed by

Herdman and Boyce[1] in their study of the distribution of copper in the oyster.

For all the reactions the tissue sections were prepared in the usual way by dissolving out the paraffin in xylol, washing with decreasing strengths of alcohol, and finally with water. In the first two reactions, namely, that produced by ammonium sulphide and ferrocyanide, a drop of the reagent flowed over the section almost instantaneously develops color. The excess of the reagent may then be washed off and the section dehydrated, cleared, and mounted in the usual way. With hæmatoxylin, a dilute aqueous solution of the pure salt is used — about 0.025 per cent — and the slide immersed in the solution for from five to fifteen minutes. Where copper is present even in very minute amounts a dark blue or slate color results. The hæmatoxylin slide may also be washed and made permanent, as above.

Finally, the reaction with the hydrobromic acid solution can best be produced by introducing a drop of the solution under the cover glass and observing the development of the stain as it takes place. The presence of copper is shown by the sudden intense violet color of the reaction. This at once begins to fade and to diffuse throughout the tissue, while the destructive effect of the acid soon obliterates any definite localization of the metal.

The results obtained by these various color reactions are identical. (1) The intestinal mucosa and its immediate sheath of muscle fibres and connective tissue are entirely free from copper, or stain very slightly in the meshes of the connective tissue where blood was probably present. (2) The entire liver tissue proper, including the secreting cells, granules, and granular secretion present in the lumina of the tubules, shows a medium stain for copper. The metal is evidently distributed throughout the cells in small amounts with considerable uniformity. It is plain from this that we have no distinct cells among the secreting epithelium whose function it is to store up copper, in a manner analogous to the calcareous cells of Barfurth. (3) The interstitial connective tissue, with the exception of the pigment cells, stains uniformly and to about the same depth of color as the secreting epithelium. (4) The pigment cells of the connective tissue stain very deeply, showing that they contain a comparatively large amount of copper. These pigment cells serve undoubtedly as depots for the storing up of this metal, and their dark green color is perhaps due to the metallic compounds contained in them.

[1] HERDMAN and BOYCE: Report of the Thompson-Yates Laboratories, Liverpool, 1899, ii.

It seems significant of the close relation which must exist between these pigment cells and the copper of the blood, that wherever blood vessels or blood sinuses are found in the liver tissue, these pigment cells are most abundant; and that where there seems to be no free passage of blood possible, few of the pigment cells are located. Thus in the very open network of connective tissue lying between the stomach and the liver proper, and in the extension of this tissue into the interior of the organ, the blood flow is fairly rapid and pigment cells are more abundant than anywhere else in the liver.

It is quite probable that the liver performs the double function of hæmolysis and hæmopoiesis, and that the pigment cells serve to store up a supply of copper for the latter process just as the vertebrate liver retains iron. The presence of copper generally in the liver may indicate a similar reserve supply, or it may be a provision for cellular respiration in an organ where rapid blood supply seems impossible from its structure.

Tissue stain for zinc. — The histological localization of zinc was found to present a much more difficult problem than that of the copper. Zinc forms few colored salts, and those which it does form are neither very characteristic nor insoluble. For example, the precipitation of chromate of zinc in the tissue was tried without success, inasmuch as the amount of washing required to remove the yellow stain of the reagent was sufficient to remove any zinc chromate that may have been formed in the tissues. The nearest approach to a satisfactory solution of the problem was found to be the reaction with nitroprusside mentioned before. Zinc nitroprusside was formed in the tissues, the excess of the reagent removed by washing, and then the well-known reaction of an alkaline sulphide with a nitroprusside used to develop a stain wherever insoluble zinc nitroprusside was present.

The method of procedure was as follows: a section, washed in the way outlined above for copper reactions, was digested with a 10 per cent nitroprusside solution at 50° C for about fifteen minutes. After cooling and standing some time, the section was washed in cold running water for fifteen minutes, — a period shown by experience with sections of other tissues free from zinc to be sufficient to remove all traces of the soluble sodium nitroprusside. At the same time washing for this length of time was not sufficient to remove the less soluble zinc salt from the tissues, if any had formed. The sections were therefore covered and a drop of potassium sulphide solution introduced beneath the glass and allowed to diffuse slowly. The instantaneous

development of the intense purple color characteristic of the reaction between a nitroprusside and an alkaline sulphide showed the presence of the former salt in the tissues. The suggestion at once arises that at least a part of this reaction is due to insoluble zinc nitroprusside. It may also be due in part to copper. That it is not entirely due to copper is plain by a comparison of two sections. If copper alone were responsible for the color, we should expect to find a uniform stain of only medium depth over the secreting tubules and connective tissue, with an intense staining of the pigment cells. As a matter of fact the pigment cells stain lightly or not at all — probably because the copper is in combination of too stable a character to be decomposed by the reagent. The connective tissue stains with medium depth, and the secreting epithelial cells are colored deeply. Evidently the intensity of the stain in the tubules cannot be accounted for on the hypothesis that it is produced by copper, since there we have the minimal amount of that metal. Further, the connective tissue about the tubules should stain equally brilliantly if the reaction were due to copper. Zinc is therefore presumably present in the epithelial cells of the tubules in abundance, and in somewhat smaller amount in the surrounding connective tissue; it is absent, or nearly so, from the pigment cells. At the same time, with the reaction so fleeting, the color compound quickly diffuses and the reaction becomes masked, so that this test alone could hardly be taken as conclusive proof that zinc is definitely localized in the secreting elements of the gland. It is possible to check and confirm this suggestion by another method. Since the two metals, copper and zinc, are located for the most part in different tissues, it should be possible to effect a separation of the connective tissue with its pigment cells rich in copper from the epithelial cells having high content of zinc.

Distribution of the metallic elements in the tissues. — It was found to be quite possible to make this separation by mechanical means. If the fresh livers are ground up in a mortar and extracted several times, the connective tissue remains in stringy masses, while the cell proteids of the epithelium are in suspension or solution. The separation can then be made by straining through cloth. Such a separation at best cannot be complete; for portions of the cells will remain with the connective tissue, and small fragments of connective tissue and pigment cells will strain through and contaminate the cellular extract. However, the results are sufficiently striking to confirm the evidence of the preceding staining methods and show how largely the two

metals are retained in different tissues. The two fractions of the liver were dried and ashed, and analyses of the ash were made for copper and zinc as before. The two ash samples themselves show most striking differences; the ash from the cells is lightly colored or nearly white, while that from the connective tissue is black and heavy, suggesting large amounts of copper oxide. The results are summarized in the following tables:

TABLE III.

DISTRIBUTION OF ASH IN LIVER TISSUE.

Part of tissue used.	Weight of dry tissue.	Proportion of whole tissue.	Ash.	Percentage of ash in tissue.	Proportion of total ash.
	grams	per cent	grams	per cent	per cent
Connective . . .	16	27.1	2.2	13.7	28.7
Glandular	43	72.9	5.45	12.6	71.3
Total	59	100.0	7.65	....	100.0

From the table it is seen that the percentage of ash in each tissue is about the same — 13.7 and 12.6 per cent respectively — but the amount of gland material is much greater than that of the connective tissue, so that two-thirds of the entire liver mass is glandular and correspondingly two-thirds of the ash of the liver is derived from it. In other words, the gland cells contain about two-thirds of the

TABLE IV.

ANALYSES OF LIVER ASH.

Tissue.	Ash.	Copper.	Copper.	Average.	Zinc.	Zinc.	Average.
	grams	grams	per cent	per cent	grams	per cent	per cent
Connective . .	a. 1.0117	0.2674	26.43	26.5	0.1004 ZnO	7.90	8.49
	b. 1.1853	0.3149	26.56		0.1342 "	9.09	
Glandular . .	a. 1.1544	0.0376	3.25	3.11	....	....	15.65
	b. 1.7048	0.0507	2.98		0.2693 ZnS	15.65	
Connective . .	a. 0.8208	0.1724	21.01	21.05	0.0921 "	7.53	8.51
	b. 0.8343	0.1760	21.09		0.1180 "	9.49	
Glandular . .	a. 1.0174	0.0372	3.65	3.34	0.2531 "	16.69	16.97
	b. 0.6160	0.0187	3.04		0.1584 "	17.26	

inorganic salts stored in the liver, while the connective tissue contains about a third.

Copper was estimated electrolytically in each case; in one sample by the rotating cathode method of Gooch and Medway. Iron was determined by permanganate titration in the usual way; while zinc was separated as the sulphide from a formic acid solution of sufficient

TABLE V.

SUMMARY OF RESULTS.

Tissue used.	Proportion of entire liver.	Ash content of each part.	Proportion of total liver ash in each fraction.	Distribution of ash per 100 gm. dry liver.	Copper in ash.	Distribution of copper in 100 gm. dry liver.	Proportion of total copper of liver in each fraction.	Copper present in each tissue.	Zinc in ash.	Distribution of zinc in 100 gm. dry liver.	Proportion of total zinc of liver in each fraction.	Zinc present in each tissue.
	p. c.	p. c.	p. c.	gm.	p. c.	gm.	p. c.	p. c.	p. c.	gm.	p. c.	p. c.
Connective	27.1	13.7	28.7	3.71	26.5	0.983	77.5	3.63	7.09	0.263	15.46	0.97
					21.05	0.781	72.1	2.88	8.51	0.386	16.85	1.16
Glandular	72.9	12.6	71.3	9.10	3.11	0.286	22.5	0.39	15.65	1.438	84.54	1.97
					3.30	0.303	27.9	0.41	16.97	1.559	83.15	2.14
The entire liver contains 12.9 per cent ash.												

strength to prevent precipitation of iron, and weighed either as the sulphide or, after solution and reprecipitation with sodium carbonate, as the oxide.[1] Tables IV and V summarize the results of the analyses of the two tissues.

By averaging the amounts of copper and zinc found in the fractions, we have calculated the quantities present in the entire livers from which they were taken, and compared these data with an analysis of whole livers collected at about the same time as a check. This comparison is tabulated in Table VI.

Finally, to complete our data the nitrogen of the two fractions has been determined and the ratio to copper and zinc obtained. The results are tabulated in Table VII.

In summarizing the results of these analyses the evidence obtained by the staining processes have been confirmed by the analytical data. The liver is found to contain considerable amounts of copper and zinc,

[1] Cf. HAMPE: Chemiker Zeitung, 1885, ix, p. 543.

both undoubtedly in some proteid combination analogous perhaps to the iron combinations found in the vertebrate liver. Both metals are present throughout the organ in small amounts, but the bulk of each is quite definitely localized in particular structures. Thus while about

TABLE VI.

A Comparison of the Analyses of the Entire Liver with the Sum of the Fractions.

	Ash.	Copper.		Zinc.	
		In ash.	In tissue.	In ash.	In tissue.
	per cent	per cent	per cent	per cent	per cent
Entire liver . . .	13.0	9.4	1.22	12.4	1.61
Sum of fractions .	12.9	9.1	1.18	13.9	1.79

one-third of the total copper is found in the gland cells, and two-thirds in the connective tissue the relative concentrations of each in individual connective tissue cells are much greater than this proportion, for the reason that nearly two-thirds of the liver mass is composed of gland cells. That is, since the content of ash in both tissues

TABLE VII.

Nitrogen Ratios.

Part of liver.	Nitrogen.	Copper (average).	Zinc (average).	Cu : N.	Zn : N.
	per cent	per cent	per cent		
Connective tissue . .	10.19	3.2	1.0	1 : 3.1	1 : 9.5
Glandular tissue . .	11.37	0.4	2.0	1 : 28.1	1 : 5.5

is practically the same — about 13 per cent — the relative concentrations in a given mass of liver bear approximately the same relation to each other as the content of the metal in the ash. In other words, in the same weights of the tissues the ratio of the amounts of copper is about 25 to 3. A single pigment cell carries eight times the amount of copper contained in a gland cell of the same mass.

In the case of zinc the same holds true; but the concentrations approximate each other much more nearly. Thus, while the gland cells as a whole carry about five times the amount of zinc found in

the connective tissue, a single gland cell contains only about twice the zinc held by a similar connective tissue mass.

Metallic compounds of proteids. — Some notion of the actual amount of copper and zinc present in the liver tissues may be had by comparing the analyses above with the amounts of metallic elements which combine with and precipitate proteids. In the work of Chittenden and Whitehouse[1] various purified proteids were precipitated by salts of the heavy metals, and the washed precipitates dried and analyzed. The precipitates produced by adding a copper salt to albumin under different conditions show three types of compounds containing 1.29, 0.96, and 0.77 per cent of copper respectively. The amount of copper present in the gland cells — about 0.4 per cent — is thus seen to be much less than that in the three insoluble copper combinations quoted above; but the amount present in the pigment cells, on the other hand, — about 3.0 per cent, — is much greater than in the richest metallic proteid compound obtainable by precipitation with copper salts.

In the case of the zinc compounds, the connective tissue contains an amount of that element corresponding very closely to that of the precipitated albumin of Chittenden and Whitehouse, namely, 0.99 per cent. The glandular tissue, however, contains twice the amount present in the richest zinc-albumin precipitate.

It is evident from these figures that we are dealing with compounds in the liver having different properties from those of familiar proteids. Possibly the nucleoproteids of this gland are rich in zinc and copper, and we have some tentative evidence to confirm this view. Until further data have been obtained, however, the discussion of this point will not be attempted.

Origin and significance of copper and zinc. — As to the origin and significance of these two elements in the tissues, much remains uncertain. Copper is undoubtedly obtained from the food of Sycotypus. The oyster, clam, and many other molluscs and crustacea which constitute the food of this animal contain copper in quite comparable amounts. The work of Henze[2] upon the hæmocyanins may be mentioned here as the authority for this statement; and his findings have in several instances been verified and extended in this laboratory.

[1] CHITTENDEN and WHITEHOUSE: Studies from the Laboratory of Physiological Chemistry, Yale University, 1885–1886, ii, p. 95.

[2] HENZE: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 370; 1904, xliii, p. 290.

But in the case of zinc the explanation is much less patent. In our examination of various common marine animals — Urosalpinx cinerea, Mytilus edulis, Modiola plicatula, Argina pexata, Eupagurus pollicaris, Ostrea virginiana, and Cancer irroratus — no appreciable amounts of zinc could be detected, with the exception of Urosalpinx, in which traces of the metal seemed to be present usually. At the same time the limited amounts of material, and the uncertainty of the qualitative methods, where minute traces of zinc are present with copper, iron, calcium, magnesium, and phosphoric acid, would not exclude the possibility that minute amounts of the element are present in the tissues of the common food material of Sycotypus. Indeed since sea water itself has been shown by Dieulafait [1] to contain about 0.002 gram of zinc per cubic metre, it is highly probable that all marine animals may contain the element in their tissues. In any case Sycotypus must exercise a selective power such that the zinc of the food is retained and stored in the liver until it reaches a maximum in the adult animal represented by the preceding analyses. The zinc must eventually come from the sea water itself; but it is more in accord with other examples of the storing up of unusual elements by animals, to suppose that the element is first picked out and retained by some simple organism or plant, and thence reaches Sycotypus in its food through a series of more complex and larger forms. Such a selective activity is seen, for example, in the storing of iodine in the tissues of certain seaweeds, and also in the gorgonian corals; in the latter case the iodine is in all probability obtained from food, while in the former it is picked up directly from the sea water.

Finally, the real significance of the zinc and copper in the liver of Sycotypus is probably to be looked for in the constant occurrence of these elements combined in the blood. Zinc is perhaps an essential element of the respiratory proteid of this mollusc and the allied Fulgur carica. Such occurrence and function is not at all surprising in view of the well-known diversity exhibited by various molluscs in this particular. It is merely an extension of the number of elements already known to be utilized by the molluscs in the performance of their respiratory exchange. The presence of zinc in the liver is thus of secondary importance; for that organ acts regularly as the storehouse for reserve elements required in the processes of hæmopoiesis and respiration.

[1] DIEULAFAIT: Comptes rendus, 1880, xc, p. 1573.

SUMMARY.

The following inorganic constituents can be recognized as normal and constant constituents of the liver of Sycotypus: copper, zinc, iron, calcium, magnesium, and phosphorus. The presence of the first two is of especial interest.

Copper comprises about 8 per cent of the total ash, or 1.2 per cent of the dry tissue. Zinc comprises about 15 per cent of the ash or 1.7 per cent of the dry tissue. Both metals can be identified in their combination in the tissues by appropriate color reactions. Copper is found uniformly distributed in small quantities — about 0.4 per cent — in the gland cells and connective tissue, but is present in especially high percentages in the greenish pigment cells — averaging about 3 per cent of the dry tissue. Zinc is found especially in the gland cells, to the amount of about 2 per cent; in a connective tissue to the extent of 1 per cent.

The liver compounds are richer in these metals in certain cases than the insoluble proteid combinations formed by the precipitation of albumin with metallic salts.

The copper and zinc are probably obtained from the food of Sycotypus, and retained in the liver as reserve material for the processes of hæmopoiesis. Both copper and zinc are present in combination in the blood, forming a respiratory proteid peculiar to this animal.

THE EFFECTS OF INTRAVENOUS INJECTIONS OF BONE MARROW EXTRACTS UPON BLOOD PRESSURE.

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INTRAVENOUS injections of animal tissue extracts, so far as we know, invariably alter the blood pressure. The most striking example is an extract of the suprarenal capsule. It was shown by Oliver and Schäfer,[1] in 1894, that a very small quantity of an aqueous extract of the medullary substance of this gland, when injected intravenously into an animal produced a great rise of arterial pressure. This problem has been worked upon by many other investigators; a partial list of the more prominent ones has been given in the bibliography.[2] It seems to be firmly established that the extract of the gland, or its active principle, adrenalin chloride (Takamini), or epinephrin (Abel), produces a marked increase in the arterial pressure, mainly by contracting the arterioles. This occurs whether the vagi are intact or divided, but is more marked in the latter case.

The effects of extracts of the pituitary body, while perhaps less striking than those of the suprarenal capsule, are not less interesting. Important work upon this subject has been done by Oliver and Schäfer,[3] Schäfer and Vincent,[4] Syzmonowicz,[5] and Howell.[6] Extracts of the hypophyseal portion of the gland do not affect blood pressure.

[1] Oliver and Schäfer: Journal of physiology, 1894, xvi, p. 1.

[2] E. v. Cyon: Archiv für die gesammte Physiologie, 1896, lxiv, p. 97: Cybulski: Wiener medicinishe Wochenschrift, 1896, xlvi, p. 255; Syzmonowicz; Zentralblatt für Physiologie, 1899, xii, p. 599; Boruttau: Archiv für die gesammte Physiologie, 1899, lxxviii, p. 97; Salvioli and Pezzolini: Archives italiennes de biologie, 1902, xxxvii, p. 380; S. J. and Clara Meltzer: This journal, 1903, ix, p. 147.

[3] Oliver and Schäfer: Journal of physiology, 1895, xviii, p. 276.

[4] Schäfer and Vincent: Journal of physiology, 1899, xxv, p. 87.

[5] Syzmonowicz: Archiv für die gesammte Physiologie, 1896, lxiv, p. 97.

[6] Howell: Journal of experimental medicine, 1898, iii, p. 245.

The weight of evidence indicates that the infundibular lobe of the pituitary body contains two substances, which have an effect upon the blood pressure when injected intravenously. One has a pressor and the other a depressor action. They differ in their solubilities in alcohol, ether, and salt solution, and by taking advantage of this they can be separated. The exact method by which these substances effect the alteration in arterial tension is not definitely known, but it probably is mainly due to their effect upon the arterioles.

Chief among those who have studied the action of extracts of nervous tissue are Schäfer and Moore [1] and others whose names are given in the bibliography.[2] The extracts produce a fall in arterial pressure before and after division of the vagi and after atropin. The gray matter seems to be richer in the active material than the white. Halliburton [3] says the fall of blood pressure is partly due to the effect on the heart, and partly to the dilatation of peripheral vessels. As to the chemical nature of the substance producing the fall, little is known. Halliburton says that all of his results can be explained on the assumption that cholin is the active agent. Vincent and Cramer [4] are of the opinion that cholin is not the active agent, but that there are two groups of substances in watery extracts, which when injected into the veins of an animal lower the blood pressure. Both are soluble in normal saline solution. One substance is readily soluble, and the other practically insoluble, in absolute alcohol. There is some evidence of the presence of a pressor substance in nervous tissue.

Extracts of the thyroid, parotid, and submaxillary glands have been shown by Oliver and Schäfer [5] to have a depressor action when injected intravenously. Also spleen extracts produce a fall of arterial pressure; but it is followed by a gradual rise, which later returns to normal. Vincent and Sheen [6] obtained with extracts of spleen tissue only a fall in arterial pressure. The former observers used glycerin and watery extracts, made at 38° C., while the latter used saline decoctions. The latter have also shown that there is a depressor

[1] Schäfer and Moore: Journal of physiology, 1896, xx, p. 26.

[2] Halliburton: Journal of physiology, 1900, xxvi, p. 229; Gulewitsch: Zeitschrift fur physiologische Chemie, 1899, xxvii, p. 50; Ott: Journal of physiology, 1900, xxvi, p. 229; Cleghorn: This journal, 1899, ii, p. 471; Vincent and Osborne: Journal of physiology, 1900, xxv, p. 283.

[3] Halliburton: Journal of physiology, 1900, xxvi, p. 229.

[4] Vincent and Cramer: Journal of physiology, 1904, xxx, p. 143.

[5] Oliver and Schäfer: Journal of physiology, 1895, xviii, p. 277.

[6] Vincent and Sheen: Journal of physiology, 1903, xxix, p. 242.

substance present in saline extracts of liver, kidney, muscle, ovaries, pancreas, lung, and intestine. These investigators also find a pressor substance in liver, spleen, and kidney. It is more soluble in cold salt solution than the depressor, and is either destroyed by boiling, or sufficient depressor substance is extracted in boiling solutions to mask the pressor effect. Some years after Brown-Séquard [1] made public the result of certain experiments upon himself, with subcutaneous injections of the expressed juice of testes of dogs and guinea-pigs, Dixon [2] studied carefully the effects of orchitic extract upon arterial pressure. He showed that the extracts produced a fall in blood pressure after a comparatively long latent period. The effect is due chiefly, he claims, to cardiac inhibition; but vaso-dilatation is one factor. The recovery is slow, gradual, and inversely proportional to the amount injected. Respiration is increased with small doses, but large doses cause a temporary cessation of respiratory movement. Cardiac and respiratory effects are absent if the vagi have been divided.

In summarizing the literature it is seen that in a few cases, as in the suprarenal capsules, the pituitary body, the liver, the spleen, and the kidney, the extracts contain a pressor, while practically all extracts contain a depressor substance.

The present work was suggested by the fact that no mention was found of the action on blood pressure of intravenous injections of bone marrow extracts. We have tried to ascertain what effects sodium chloride solution extracts of bone marrow produced upon blood pressure in dogs before and after section of the vagi and after the use of atropin. We also tried to determine roughly the temperature at which the active principle is most readily soluble, and whether or not it is destroyed by boiling. We had in mind also to study the action of marrow extracts of various species on the blood pressure in a single species, and whether the effect of these extracts was specific; *i. e.*, is the action of the extracts made from one species the same when injected into different species? Our data on this point are too scanty as yet to warrant any conclusions. We also made a few experiments with the view of determining whether or not there was any direct antagonism between the depressor substance of marrow extracts and adrenalin chloride. This was suggested by the work on the antagonism between the depressor substance of the pituitary body and adrenalin

[1] Brown-Séquard: Archives de physiologie, 1889, xxi, p. 651.

[2] Dixon: Journal of physiology, 1901, xxvi, p. 244.

chloride, which Hamburger[1] carried on under the direction of Professor G. N. Stewart. In addition it is our intention to attempt to isolate more definitely the active depressor principle.

Methods.

The extracts employed were prepared from the marrow of the long bones of dog, sheep, and ox, and therefore consisted mainly of the yellow marrow. The extracts were all made with 0.9 per cent solution of sodium chloride. Different methods were used in making the extracts and, for the sake of convenience, for the solutions we have adopted names which will be used throughout the paper.

1. To marrow removed from fresh bones sodium chloride solution was added in the proportion of 1 c.c. per gram of marrow, and the two thoroughly mixed in a mortar. Sometimes sand was employed to facilitate the mixing. The mixture was heated to about 40 C. and filtered through cheese cloth. After standing a few minutes the fat rose to the surface and was removed. This preparation, which was now ready for use, we termed "**extract filtered warm.**" It was demonstrated in several experiments that the extract could be passed through paper without having its action altered.

2. When the above extract was allowed to stand in the cold for some time, a grayish sediment settled out and could be filtered off. This filtrate was fairly clear and of a red color. It was now ready for use. It was termed "**extract filtered cold.**"

3. The sediment remaining upon the cheese cloth from the above was mixed with sodium chloride solution, the latter being added until the volume of the mixture was the same as before the removal of the filtrate. This was then heated to 40° C. and filtered through cheese cloth. This preparation was called "**sediment extract.**"

4. The marrow was heated until the fat was liquefied; the latter was then filtered off. The precipitate remaining upon the cheese cloth was then extracted with boiling sodium chloride solution and again filtered. This solution was termed "**extract made by boiling.**"

Dogs were used for testing the solutions. The anæsthetic employed was ether. The blood pressure was recorded from the carotid artery. The rate of the pulse and respiration was determined during the course of the experiment by the aid of a stop watch. Tracings of the respiration were obtained by a trachea tube and tambour. The injec-

[1] Hamburger: This journal, 1904, xi, p. 282.

TABLE

EXTRACTS FROM

Exp. No.	Wt. of dog.	First injection.	Vagi.	Blood pressure.			Time to return to normal.
				Normal.	Rise.	Fall.	
	lbs.			mm. Hg.	mm.	mm.	secs.
1	18	Dog marrow, "extract filtered warm" . .	intact	110	114	84	180
2	12	Sodium chloride . . .	"	132	135	132	13
3	20	Ox marrow, "extract filtered warm" . .	"	123	124	95	240
4	21	Sheep marrow, "extract filtered warm" .	"	112	123	112	90
5	15	Ox marrow, "extract filtered cold" . . .	"	77	86	77	60
6	17	Ox marrow, "extract filtered warm" . .	"	120	132	55	900
7	25	Ox marrow, "extract filtered warm" . .	"	89	93	64	210
8	16	Sodium chloride . . .	"	134	134	134	....
9	21	Ox marrow, "sediment extract"	"	82	83	36	900
10	19	Ox marrow, "extract filtered warm" . . .	"	108	112	96	300
12[2]	39	Ox marrow, "sediment extract"	"	117	125	40	270
13	16	12 c.c. adrenalin chloride	"	77	94	77	26
14	20	25 c c. adrenalin chloride	"	120	165	120	65
15	23	Ox marrow, "made by boiling"	"	139	146	74	286
16	23	Dog marrow, "extract filtered warm" . .	"	145	152	127	102
17	23	Ox marrow, "extract made by boiling" .	divided	142	149	123	25

[1] A large number of injections were made upon each dog, as a rule, and two are selected for the table.

[2] Using an induced current of constant strength, we observed that stimulation of peripheral ends of the divided vagi was more effective in the dogs not affected by goitre.

I.

PROTOCOLS.[1]

Second injection.	Vagi.	Blood pressure.			Time to return to normal.	Remarks.
		Normal.	Rise.	Fall.		
		mm.	mm.	mm.	secs.	
Same as first . .	divided	104	110	95	60	Extract weaker than the ox marrow extract.
Ox "extract filtered cold" . .	intact	117	124	117	30	Goitre dog.[2]
Ox "extract filtered cold" . .	"	120	123	118	40	This extract of first injection had been heated to 55° C. in making.
Ox "extract filtered warm" .	"	114	128	90	240	
Ox "extract filtered warm" .	"	90	98	67	260	
Same as first . .	divided	97	102	82	100	Goitre dog. Following the first injection there was great augmentation of respiration.
Same as first . .	"	91	107	84	180	
Ox "extract filtered cold" . .	intact	144	149	144		Goitre dog. Heart irregular.
Same as first . .	intact	106	113	104		Temp. of first injection 36° C.; temp. of second injection 21½° C.
Same as first . .	divided	130	139	54	33	
Ox "extract filtered warm" .	intact	82	86	73	36	The mixture[4] of doses one and two gave results as follows: normal b. p., 34; rise, 82; fall, 34; time to return to normal 100 sec.
Ox "extract filtered warm" .	intact	126	122	109	26	The mixture of doses one and two gave the following result: normal b p., 120; rise, 158; fall, 120; time, 78 sec.
Same as first . .	divided	141	147	110	52	Goitre dog.

[3] Experiment 11 was upon a vicious dog which was chloroformed with great difficulty. The effects of the injections were not constant.

[4] The extracts were mixed so that 25 c.c. of the mixture contained the active principle of both doses used separately.

tions were made by means of a burette and cannula connected with the central end of a femoral vein. The time of injecting was recorded on the drum with a signal magnet. The temperature of the solutions as injected was taken from a thermometer inserted into the tubing leading from the burette to the cannula. Injections for the most part were made at approximately body temperature, and may be so considered unless otherwise stated. For control experiments the sodium chloride solution in the same concentration as that employed in making the extracts was injected.

Experimental Results.

"**Extract filtered warm.**" *Dog marrow.* — This marrow is of a deep red color and of a soft consistence at room temperature. The injection of 3 c.c. of the "extract filtered warm" into the veins of an eighteen-pound dog produced a slight fall in the blood pressure. The use of a 25 c.c. dose produced a sudden small rise followed by a sharp and pronounced fall. These phenomena were observed in all subsequent experiments, with a few exceptions which will be mentioned in the proper place. The "extract filtered warm" solutions did not produce as deep and lasting a fall as that of Fig. 1, which was caused by the "sediment extract." After section of the vagus nerves the extract produced the same effect upon blood pressure, with the exception that the fall of pressure was not so low, nor did it last so long a time. The injection of an equal dose of sodium chloride solution produced a sudden small rise in pressure, which immediately returned to normal. In no case did we ever get a fall in blood pressure with the sodium chloride solution. The rise from the sodium chloride injection seemed to be of the same height as that produced by any of the injections of the "extract filtered warm" solution of dog marrow.

Sheep marrow. — The appearance of the marrow from the bones of sheep is somewhat like that from the bones of the dog, but it is not so red; it apparently contains more fat. The exact concentration of this solution was not determined, as only small quantities of the marrow were obtainable. However, the concentration was probably much weaker than those employed in most of our experiments. An injection of 25 c.c. of the "extract filtered warm" into a fifteen-pound dog produced a rise in blood pressure, which was not succeeded by a fall below normal. The rise, however, was greater than that produced by an equal volume of sodium chloride solution alone, and

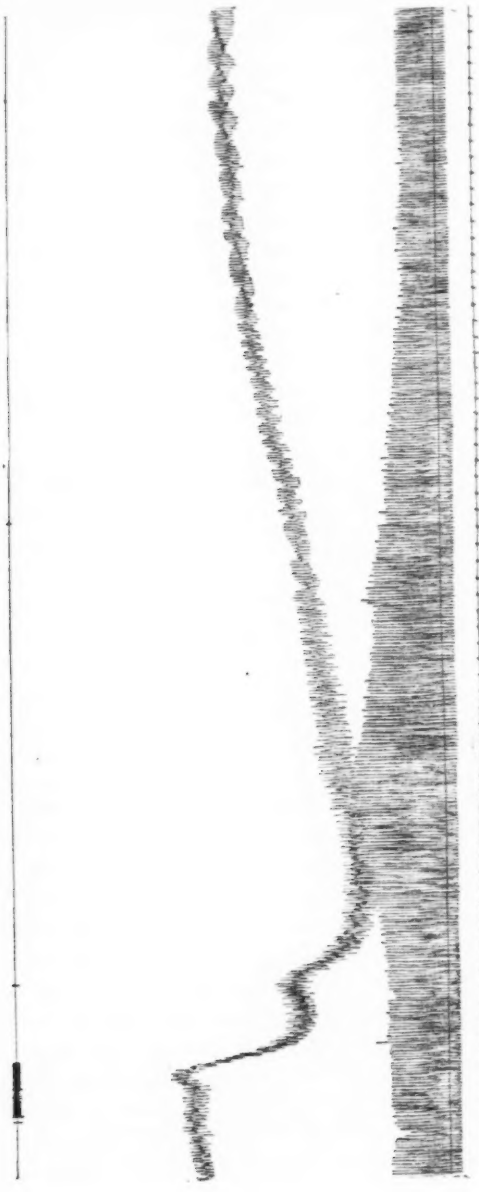

FIGURE 1. — Experiment 12. Adult bitch. Weight 39 lbs. Vagi intact. The order of the tracings is from above downward, the signal, blood pressure, respiration, abscissa, and time. The respiratory tracing overlaps the abscissa. The time is in half seconds. The signal indicates, in addition to the time of injection, the end of the ten-second period counts of pulse and respiration. This was the first injection of this dog, and consisted of 50 c.c. ox marrow "sediment extract."

lasted for a longer time, but came gradually back to normal in the course of a few seconds. Very little weight can be given to this result, as only one experiment was performed, for the reason given above.

Ox marrow. — This marrow differs in appearance from both the dog and sheep marrow. It is white or yellowish in color, being usually of a firm consistence at room temperature. In some cases we obtained considerable quantities of the yellowish-colored marrow, which was invariably of a softer consistence than the white marrow. For our experiments, however, all of the marrow in the shafts of the long bones was mixed and extracted together. It was found that a 5 or 10 c.c. dose of this always produced a slight fall in blood pressure. In order to get decisive results 25 c.c. were usually injected at a time. The injection always produced a rise which was followed by a pronounced fall. This rise was frequently greater than that produced by the same quantity of saline solution. The fall was relatively long, often lasting three to fifteen minutes (Fig. 1). The heart rate was usually accelerated during the first stage of the fall, and later was somewhat slowed as the pressure returned to normal. A second injection of the same preparation usually did not give as great a fall as the first, and the period of lowered blood pressure was much shorter. After the division of the vagi, an injection of the extract always produced a fall of blood pressure. This fall was usually not so great as before the vagi were cut, and was of shorter duration (Fig. 2). After inactivation of the vagi by use of atropin — this being determined by stimulating both nerves — an injection of the extract failed to produce a fall of pressure. Further observations on this point are necessary. The extract filtered warm was injected at various temperatures. At body temperature, or somewhere near it, the typical fall was produced. Within certain limits the depressor action was directly proportional to the temperature. When the temperature was reduced to 20 to 25° C., the effect was about the same as that produced by the injection of an equal amount of sodium chloride solution, excepting in the cases where a distinct pressor effect was evidenced. As a rule, an injection of the "extract filtered warm" in addition to the effects upon blood pressure produced an increase in amplitude of respiration and not infrequently of rate also. In a few cases the rate was slightly slowed.

"**Extract filtered cold.**" — In no instance did an injection of 25 c.c. of this solution into the veins of a dog produce a fall of blood pressure.

In some cases it produced a rise slightly greater than that produced by the same amount of sodium chloride solution. When this occurred, the result was not different after section of the vagi.

"**Sediment extract.**" — This solution produced the typical fall in blood pressure (Fig. 1). In one experiment the fall amounted to 85 m.m. of mercury. The fall was not preceded by a rise any greater than would have been caused by a sodium chloride injection of the same amount. A second injection of the "sediment extract" gave the same results as a second injection of "extract filtered warm." An injection after division of the vagi gave the same results as described under "extract filtered warm" (Fig. 2).

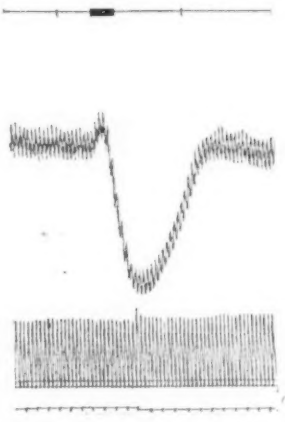

FIGURE 2. — Experiment 12. Vagi divided. Injection same as in Fig. 1; the order of the tracings is also the same.

Extract made by boiling. — This extract produced the typical fall that resulted from an injection of the "extract filtered warm" or the "sediment extract"; but there was no rise preceding the fall, or only a very slight one corresponding to the rise that would have been produced by an injection of the same amount of sodium chloride solution. A second injection of this extract also produced the same results as a second injection of the "extract filtered warm" or the "sediment extract." A fall occurred after division of the vagi the same as with the two other active depressor extracts.

Fat suspended in sodium chloride solution. — To eliminate the possibility that the depressor effect might be due to the small amount of fat necessarily present in the solutions, a small amount of marrow fat obtained in the preparation of the extracts was shaken with warm sodium chloride solution, and injected. The results were the same as for sodium chloride solution alone.

Antagonism of adrenalin chloride to the depressor substance. — There is a physiological antagonism existing between these two substances. The action of the one which is present in the larger pharmacological dose will predominate when a mixture of the two is injected. The dosage may be so graduated that the adrenalin rise will precede the

fall produced by the marrow extract. This occurs only when the adrenalin dose is small and the other is large. The curious phenomenon was observed that when the dosage of each was such as to produce distinct effects — but the adrenalin rise being greater than the marrow fall — the mixture of the two doses produces a rise less in height than the adrenalin alone, but of much longer duration.

Summary and Discussion.

There is contained in the marrow of long bones a substance which when mixed with sodium chloride solution and injected into the veins of a dog produces a fall of blood pressure. This is true of the marrow of ox and dog and probably other animals. The cause of the fall is probably chiefly a vaso-dilatation. It comes on shortly after the injection; the heart beat during the fall is not decreased in frequency or force. In fact the rate is usually increased on the downward curve, and slightly decreased as the curve approaches the original level. These facts are in harmony with the usual statements regarding the depressor effects of most organ extracts; *i. e.*, the depression of the blood pressure is usually due to vaso-dilatation. The fact that a second injection does not produce as great a depression as the first is in accordance with what is known regarding the action of an extract of the pituitary body. The fact that the fall occurs when the injection is made after section of the vagi is further evidence that it is due to vaso-dilatation. The results also indicate that the marrow extracts which have not been heated far above body temperature contain a pressor substance. This harmonizes with the fact before stated regarding the pressor substances of liver, spleen, and kidney. The marrow extracts contain in most cases a substance which stimulates respiration. Regarding the nature of the active substance, it has been shown that it is insoluble or only slightly soluble in cold, and that it is soluble in warm salt solution and that it is not destroyed by boiling. The solution containing the active substance, even after passing repeatedly through filter paper, is not clear, and does not become clear by sedimentation. Table I is from extracts of the protocols of all experiments done, and gives the most important results of each experiment.

AUSCULTATION OF THE RHYTHMIC SOUNDS PRODUCED BY THE STOMACH AND INTESTINES.[1]

By W. B. CANNON.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

IN reporting, in 1902, observations on the movements of the intestines, I made note[2] of an instance of rhythmic sounds accompanying the movements of rhythmic segmentation in the small gut. It occurred to me at that time that the sounds heard over the abdomen might indicate the mechanical activities going on in the alimentary canal in man, but only recently has my attention been strongly aroused to the interest and possible practical value of abdominal auscultation. The loud gurgling sounds produced by the intestines were, of course, observed and recorded centuries ago — the descriptive designation "borborygmus" was employed even by Hippocrates. And Robert Hooke, in a remarkable passage written more than a hundred years before Laennec, suggested "that it may be possible to discover the Motions of the Internal Parts of Bodies . . . by the sound they make, that one may discover the works performed in the several Offices and Shops of a Man's Body, and thereby discover what Instrument or Engine is out of order, what Works are going on at several Times and lie still at others"; and in support of this idea Hooke mentioned, among other instances, the hearing of the "Motion of Wind to and fro in the Guts."[3] The suggestion that abdominal sounds may be useful in discovering the works of the stomach and intestines has, however, received but scant attention. In 1849 Hooker published an essay[4] in which he described variations in the

[1] The first results of this investigation were reported at the meeting of the American Gastroenterological Association, April 24, 1905. See Medical News, New York, May 20, 1905.

[2] CANNON: This journal, 1902, vi, p. 259.

[3] HOOKE: Posthumous works, London, 1705. The method of improving natural philosophy, pp. 39 and 40.

[4] HOOKER: Boston medical and surgical journal, 1849, xl, pp. 409, 439.

frequency and intensity of intestinal gurglings in the course of different diseases of the digestive organs. Since that time other writers have classified the sounds normally audible into splashings, rattling or rustling noises, the transmitted murmurs of respiration, and the rhythmic pulsation of the aorta.[1] These sounds, however, according to L. Bernard,[2] are not constant over the abdominal organs, nor do the vibrations heard characteristically in the healthy individual alter in pathological conditions.[3] Even in the most recent and most complete treatises on auscultation [4] the only additional statements, so far as the gastroenteric tract is concerned, are with regard to the rubbing noises audible in cases of inflammation, and the piping notes that can be heard when there is intestinal stenosis. Any further notice of the facts or possibilities of auscultation of the stomach and intestines during digestion I have been unable to find.

As any one can easily determine, the abdomen is not poor in noises; on the contrary, it is usually much richer than the thorax, and the noises are of the most diverse character, from soft gurglings to loud rumbling explosions. Any special attention to the peculiarities of certain sounds in the general tumult audible at the height of digestion was hardly to be expected, so long as the nature of the motor activities of the stomach and intestines was not well understood. Within a few years our knowledge of these activities has been largely augmented. This advance should enable us to recognize more accurately the relation between the movements of the alimentary canal and the sounds these movements produce.

The most characteristic feature of the movements of the stomach and intestines, as observed in the lower animals, is without doubt *rhythmicity*. Over the pyloric end of the stomach, during gastric digestion,[5] peristaltic waves are constantly passing in rhythmic succession; in the small intestine the most usual activity is a repeated segmentation of the food by rhythmic contractions of the circular muscle,[6]

[1] See WINKEL: Jahresbericht der Gesellschaft für Natur- und Heilkunde in Dresden. Sitzung, December 6, 1873.

[2] BERNARD, L.: Zur Auscultation des Abdomens. Inaugural-Dissertation, Würzburg, 1879.

[3] Evidence is presented in this paper that BERNARD is mistaken in his first statement; he may be mistaken also in his second statement.

[4] EULENBERG, KOLLE, and WEINTRAUD: Lehrbuch der klinischen Untersuchungsmethoden. Berlin and Vienna, 1904, i, p. 691.

[5] CANNON: This journal, 1898, i, p. 367.

[6] CANNON: This journal, 1902, vi, pp. 256, 265.

occurring over and over again, and not causing the food notably to advance; and in the ascending colon, during short periods, anti-peristaltic waves rhythmically follow one another toward the cæcum.

The condition most favorable for the production of sounds in the alimentary canal is the presence of a gas mixed with food more or less fluid. When the food and the gas are churned together, a sound must result. Air in fine division can be introduced into the stomach by eating in combination with other food or by themselves such preparations as soufflés, light omelettes, toast, or very porous bread. I have also used a thin paste of gluten flour and milk, thoroughly stirred with white of egg until the mixture was frothy. Eaten with a little cream and sugar, this mixture is not unpleasant. These preparations should not be chewed so thoroughly as to drive much of the air from the small cells in which it lies enclosed. When such food is eaten, rhythmic sounds can be heard over the pyloric end of the stomach and later over the lower quadrants of the abdomen.

In listening to these sounds I have made use of a Bowles stethoscope with the hard-rubber disc of the metal chamber two inches in diameter. The flatness and weight of the metal chamber render it so stable that it remains where placed without being held; and by the addition of a rubber tube of sufficient length the stethoscope will reach easily to any situation on the observer's own abdomen. For several months I have kept the stethoscope at hand near my bed, and when unable to sleep I have used it in listening to the sounds of digestion. At times in the quiet of the night it is possible to hear the sounds without the stethoscope. Indeed the vibrations are sometimes so strong that they can be felt in the abdomen, or perceived, like the tactile fremitus of the chest, by placing the hand over the region in which the sound arises.

The rhythmic sounds are not due to respiration; they differ from the respiratory murmurs in rate and time. Nor are they due, as one who hears the confusion for the first time might suspect, to the chance choice of a rate and the selection of such sounds out of the confusion as correspond to that rate. Graphic records of the sounds produced by the stomach and small intestine have been secured, and the element of human judgment thereby eliminated. In registering the sounds of digestion I have employed the first method used by Hürthle[1] to register the heart sounds. A carbon telephone transmitter, rendered specially sensitive by the use of rather coarse carbon granules loosely

[1] HÜRTHLE: Archiv für die gesammte Physiologie, 1895, lx, p. 264.

disposed, was connected in series with five dry cells (total electromotive force, 5.5 volts) to the primary coil of an inductorium.[1] The secondary coil of the inductorium was attached to platinum electrodes in a moist chamber. Over the electrodes lay the nerve of a nerve-muscle preparation. The contraction of the muscle raised a lever which wrote on a smoked drum. So sensitive was this arrangement that ordinary conversation could not be carried on near the apparatus without marring the record. Sound vibrations are conducted from one point to another in the abdomen much better than in the thorax. But when sounds not arising immediately under the transmitter caused the muscle to contract, the recording of these muffled outlying vibrations could be largely avoided by withdrawing the secondary coil of the inductorium to a proper distance. In order that the observer might listen to the sounds while they were being recorded, a telephone receiver was arranged to be thrown into circuit at will.

The Sounds Produced by the Stomach.

The active end of the stomach is the pyloric end. The food in the antrum is repeatedly compressed by peristaltic waves moving up to the pylorus. If the sphincter does not relax as the ring of constriction approaches, the only escape for the food is back through the advancing ring. Since the waves are recurring with rhythmic regularity and the pylorus relaxes only occasionally, the food near the pylorus must be squeezed and regurgitated by well-nigh every constriction ring.

That the rhythmic gastric sound is caused by the escape of the food backward through the narrow moving orifice was proved by the following observation. A mixture of starch paste, white of egg and subnitrate of bismuth, stirred with an egg-beater until frothy, was given by stomach tube to a cat. The cat's hair had been cut short over the pyloric region, and the skin wet with water. When a stethoscope was applied, little gurgling explosions could be heard at intervals of about 13 seconds. The animal was then examined with the Röntgen rays, and peristaltic waves were found recurring at intervals of 13 to 14 seconds. As a constriction was about to pass up to the pylorus, I stopped the electrical interrupter and listened. At the proper time the characteristic sound occurred. Meanwhile no food

[1] I wish to thank Mr. H. V. Hayes and Mr. G. K. Thompson of the American Bell Telephone Company for their kindness in furnishing apparatus for my use.

had left the stomach; the sounds must have been due to the regurgitation of the food back through the advancing peristaltic ring.

Since the pyloric end of the stomach reaches farther to the right and to the front than any other part, it is clear that lying on the left side of the body or on the back or between these two positions will bring the pyloric end uppermost. When the stomach is so situated, the lighter food, *i. e.*, food mixed with air, will naturally rise into the pyloric end. Peristaltic waves passing over this somewhat viscous mixture of air and chymous food will then, for reasons already stated, produce audible vibrations. Sounds quite distinct when the subject lay on his left side became very weak or inaudible when he turned so that the pyloric end was lowermost.

The stomach sounds can best be heard after a fairly bountiful meal in which has been included a large admixture of the food of spongy consistency already mentioned. The subject should lie on his left side or on his back or in any intermediate position. The disc of the stethoscope should be placed about midway between the umbilicus and the lower end of the sternum, and to the right of the median line. The sounds are usually loud, rattling, explosive, and of a characteristic quality, quickly recognized after they have once been fixed in mind. But occasionally there is only the recurrence of a short series of pops. In some individuals the sounds are louder and more distinct than they are in others; and in all the cases I have studied, the sounds, even within two or three minutes, have varied considerably in intensity. At times the characteristic explosive discharges last several seconds; at other times there is at the regular period merely a sharp, short report. Between the moments when the typical sounds return one can ordinarily hear with more or less distinctness a sudden little pop, and perhaps several, always coming at irregular intervals. These sharp pops, which resemble the bursting of bubbles, can be heard in all parts of the abdomen, but with greatest frequency on the right side.

The gastric sound recurs approximately every 20 seconds. In one individual the interval was usually 17 to 19 seconds; in another about 21 seconds, and in a third about 24 seconds. These rates vary, as the rate of gastric peristalsis in the cat varies,[1] at different times in the same individual. In the first case mentioned above for example, the interval was occasionally 20 and 21 seconds. In all lower animals, except the rabbit, that I have examined with the Röntgen rays, peristaltic waves have been found running over the

[1] CANNON: This journal, 1904, xii, p. 392.

stomach with monotonous regularity whenever, during gastric digestion, the animal has been observed. It is probable that in man also gastric peristalsis runs in continuous rhythm until the stomach is empty, for in one case observation during the first four hours after a meal revealed only occasional short interruptions of the rhythmic sounds. The sounds are likely to be thus interrupted even when they have been for some time clearly and regularly audible. The silence may cover one, two, or even three of the regular periods. It is noteworthy that when the sound can be heard again it continues the previous rhythm. This fact is illustrated by the following figures, showing the number of seconds between successive gastric sounds about two hours after dinner:

19	20
38 = 19 + 19	19
18	19
19	20
59 = 19 + 20 + 20	38 = 19 + 19
19	20

The equations show that the normal periods have been preserved; it is probable, therefore, that the peristaltic rhythm has been continuous, although each wave has not produced a sound. The sound just previous to a silent interval is likely, in my experience, to be somewhat louder and more prolonged than is usual. It may be that this prolonged sound means a discharge of food through the pylorus, and that the conditions in the antrum are thereby so altered that the immediately succeeding waves can cause no sounds until the antrum is again normally filled; but I have no evidence of this.

Fig. 1 is the copy of a record, secured by the telephone method previously described, which shows graphically many of the features of the stomach sounds above mentioned. The different heights of the separate marks indicate variations in the intensity of the sounds. The duration of the sounds also can be judged; for example, at *c* and *e* they are more prolonged than before *a*. One of the intermediate pop sounds is recorded at *a*. Silent intervals are indicated in the regions *b*, *d*, and *f*. In these regions arrows have been placed at the points where the sounds would have recorded if present. The regular rhythm is resumed in continuation of the previous rhythm. It should not be supposed that the silent intervals are always as frequent as this record shows them; I have one tracing in which the marks are

not only rhythmically regular, but of almost the same height, uninterruptedly for fifteen minutes.

The evidence that the rhythmic sounds audible over the pyloric region are due to the rhythmic recurrence of peristaltic waves moving up to the pylorus has been presented in a comparison of the conditions in man and in the cat. This evidence is confirmed by observations of Moritz on himself. He introduced a stomach tube into the pyloric end of his stomach, and found that there were rhythmic oscillations of the intragastric pressure in that region. Examination of his records proves that the rate of gastric peristalsis, in his case also, is approximately three waves per minute, or waves at intervals of about 20 seconds.[1]

FIGURE 1.—Graphic record of the stomach sounds secured by placing over the pyloric region a telephone transmitter activating a nerve muscle preparation. The time is marked in seconds.

THE SOUNDS PRODUCED BY THE SMALL INTESTINE.

As stated in the report of a previous investigation rhythmic segmentation, although not always present even when the intestinal contents are abundant, is by far the most common mechanical process to be observed in the small gut.[2] The process has been studied not only in the cat, but also in the dog and rat. In the rabbit the circular muscles are rhythmic in action, but they have not been found segmenting the food as in the dog and cat. In these animals a long mass of food is repeatedly divided into little segments by the rhythmic constrictions; in the rabbit, on the other hand, all that I have observed is an oscillation of a small mass of the food to and fro in the lumen of the gut

[1] MORITZ: Zeitschrift für Biologie, 1895, xxxii, p. 353.

[2] CANNON: This journal, 1902, vi, p. 256.

—as if a circular constriction in one place forced the mass forward, then a circular constriction in front forced it back, whereupon a return of the first constriction caused the process to be repeated, and so on at a rapid rate for many minutes.

The movements of rhythmic segmentation have a more rapid rate than the stomach movements. In the cat the peristaltic waves of the stomach occur from 4 to 6 times per minute, the segmenting movements sometimes 18 to 21, sometimes 28 to 30 times per minute; in the dog the gastric waves run a little more rapidly than 4 per minute, the segmenting movements from 12 to 14, and from 18 to 22 times per minute. In both animals the rhythmic contractions of the small intestine are from three to five times as frequent as the waves of gastric peristalsis.

Usually, if one listens over the lower abdomen, especially over the right lower quadrant, during the height of digestion, one hears what seems at first only a great confusion of noises. Without experience it is difficult to distinguish in the midst of this tumult the rhythmic sounds of the small intestine. It is better to listen in the night after the stomach is empty, or better still an hour or two before breakfast. The stomach is then producing no sounds, and the active part of the large intestine can be avoided by placing the disc of the stethoscope over the lower left quadrant of the abdomen. As already mentioned, these sounds can sometimes be heard in the quiet of the night without the use of the stethoscope. I have heard them thus and determined their rate by listening at the same time to a clock ticking twice a second.

The rhythmic sound of the small intestine is different in quality from the gushing, explosive sound of the stomach. To be sure, the intestinal sound is not always the same: sometimes it is a soft rustling of fine crepitating noises; sometimes a group of little rattling explosive discharges, as if an exaggerated crepitation; and sometimes a rough rolling rumble, like miniature thunder. But after these variations in quality there remain three features of the intestinal sounds that are quite distinctive. First, the sounds usually rise slowly to an acme of intensity and then gradually subside; but they may increase slowly to a maximum and suddenly cease, or may begin loud and then gently decrease to silence. Thus, each sound may last 2 or 3 seconds, or more. The second characteristic of the rhythm is its persistence for some time in one place; it may be audible for a minute, or it may last for many minutes, but it does not move away as the sound pro-

duced by a peristaltic wave would move. The third feature is the distinctive rate. This rate is usually one sound every 7 or 8 seconds, but I have heard the sounds 4 or 5 seconds apart, and at times 10 seconds apart. This rate would occasion from 7 to 12 movements per minute. The rhythmic contractions of the small intestine are thus from two to four times as frequent as the waves of gastric peristalsis, — a condition corresponding to that in the cat and dog (see p. 346). This fact and the fact that these rhythmic sounds can at times be heard loudest in the left flank, far from the active ascending colon, have led me to regard these sounds as a result of the activity of the small intestine rather than of the colon. Of course, at any one time there will be some variation in the rate, but usually it is not great, as the following figures, showing the number of seconds between the beginnings of successive sounds, will indicate:

8	8
8	$15 = 7 + 8$
6	9
8	6
$13 = 7 + 6$	9

As these figures illustrate, the sound sometimes skips the regular period, but continues the rhythm on reappearing.

In the morning, after an ample dinner the evening before, I have heard these rhythmic sounds continue without interruption for more than an hour and a half. The intestinal sounds are not peculiar to the morning hours, though they are most clearly distinguishable at that time. After learning their qualities and rate I have heard them distinctly in the midst of active digestion in the afternoon and evening. Nor are they peculiar to the left side of the body; at times I have heard them loudest on the right side.

In describing in 1902 the rhythmic sounds attending rhythmic segmentation in a cat with opened abdomen, I stated, "As new rings occurred the old relaxed, but apparently with tardiness, for the contents gurgled as if forced through the narrowed lumen."[1] The contraction of the circular muscle at fairly regular intervals along the length of a mass of food cuts the mass into segments, and the repeated splitting of these segments to form new segments must bring about with each operation a squeezing and shifting of the food, almost simultaneously along the whole line. If the food contains

[1] CANNON: This journal, 1902, vi, p. 259.

air, the squeezing and shifting will result in audible rumblings and crepitations. It seems probable that the presence of *valvulae conniventes* causes the sounds to be louder than they would be in a smooth intestine. The rather long duration of these sounds — sometimes 3 seconds and more — leads me to think that the process in the human body is like that observable in the cat and dog, and not the simple to-and-fro oscillation of a small bit of food observable in the rabbit.

A record of the rhythmic sounds of the small intestine, secured by the method already described, is shown in Fig. 2. It is a record of sounds heard before breakfast one morning about half-past nine o'clock. The dinner at six o'clock the previous evening consisted of

FIGURE 2. — Graphic record of the rhythmic sounds of the small intestine. The height of the records has been reduced to one-fourth the original size. The time is marked in seconds.

grape fruit, mackerel, potato, cucumber and tomato salad, four slices of bread and butter, and strawberries and cream with cocoanut cakes. About ten o'clock in the evening, four slices of bread and butter and a glass of milk were taken. At the time this record was made the telephone transmitter was placed on the lower left quadrant of the abdomen. The duration of the sounds is not indicated, since the muscle recorded in each case only at the climax of intensity.

THE SOUNDS PRODUCED BY THE LARGE INTESTINE.

The antiperistalsis of the proximal portion of the large intestine was incidentally observed in the cat by Jacobj[1] in 1890, during a research on colchicum poisoning. In 1902 this activity of the large intestine was established as the normal activity in the undisturbed animal by observations with the Röntgen rays.[2] In 1904 Elliott and Barclay-Smith[3] confirmed these observations on the cat and found that in the rat and guinea-pig, and to a slight extent in the rabbit, hedgehog, and ferret, antiperistalsis was present in the proximal

[1] JACOBJ: Archiv für experimentelle Pathologie und Pharmakologie, 1890, xxvii, p. 147.

[2] CANNON: This journal, 1902, vi, p. 265.

[3] ELLIOTT and BARCLAY-SMITH: Journal of physiology, 1904, xxxi, p. 272.

colon. They also found in the herbivorous animals they studied that sacculation of the proximal colon was associated with churning movements, each sacculus becoming at times the seat of swaying oscillations. The greater the churning activity of the proximal colon, the more marked was the sacculation of its wall. The colon of man is of the sacculated herbivorous, rather than of the carnivorous type. The sign of a proximal colon which mixes and churns its contained food is a uniform soft consistency of the contents. In the human being this condition is realized only in the cæcum and ascending colon; the contents of the transverse colon are generally as firm as those of the rectum.[1] Elliott and Barclay-Smith assumed, therefore, that in man the food entering the proximal colon "is still delayed by a backward current, still commingled by the activity of the walls of the sacculi."

Antiperistaltic waves moving toward the cæcum must press the food into a blind pouch, just as do the peristaltic waves of the stomach so long as the pylorus remains closed. The only escape for the food must be, as in the stomach, back through the advancing ring. Each peristaltic wave should produce a sound similar in quality to that of the stomach. From the analogy of the cat one would expect these waves to have about the same rate of recurrence as the gastric waves. One would expect likewise that they would run not continuously, like the gastric waves, but for short periods when new masses of food enter the colon from the small intestine; that they would appear, as in the cat, after the injection of a large enema; and that during the periods of activity the waves would follow one another in a fairly regular rhythm.

The greater activity in the right lower quadrant of the abdomen is manifested by the more frequent occurrence of sounds there than in the left lower quadrant. At times an almost constant succession of little popping noises and faint gurglings can be heard in the region over the ascending colon when the region over the descending colon is quite silent. But in spite of listening in the region of the cæcum for hours, at different times of the day, and with my body in various positions, a uniform and characteristic rhythm of the sounds in this region, if it be present, has escaped me. Sounds of a coarse rumbling character somewhat like those of the stomach, but usually more prolonged, are at times audible. These sounds were once heard recurring regularly for a short period at intervals of about 20

[1] Roith: Merkel and Bonnet's Arbeiten, 1903, xx, p. 32.

seconds. More commonly, in my experience, such irregular intervals as these — 45, 25, 35, 27, 25, 14, and 29 seconds — are observable. Inasmuch as these sounds are not clearly rhythmic it seems questionable whether they are produced in only one part of the intestine. But these gurglings are heard loudest along the ascending and transverse colon, and for that reason are probably due to activities of the large bowel.

The absence of a regular rhythm in the repeated contractions of the large intestine has been supported by experience with enemata. The enemata consisted of starch and a little flour boiled in normal salt solution. The resulting paste was thin, yet viscid enough to be stirred into a froth much like soapsuds. Enemata of this kind, made frothy, were introduced at body temperature in amounts varying between 1500 c.c. and 2000 c.c. In order to avoid confusing noises from the stomach their effects were studied in the morning before breakfasting, and they were usually preceded by a cleansing enema of warm normal salt solution. If the body is kept in a horizontal position, the fluid can be retained for a half hour or more without difficulty. During this time, especially if the pelvis is raised, there are repeated pains or cramps referred most commonly to the region of the hepatic flexure of the colon. Sometimes the pains are referred also to midway in the transverse, and less often to the ascending colon. They are very distinct and quite unmistakable in their character. It is remarkable that these recurring cramps, which are undoubtedly due to contractions of the intestine, are ordinarily not felt in the descending colon, sigmoid flexure, or rectum, but are restricted to the proximal colon, the region which in the lower animals is characterized by the greatest activity.

The contractions attending the pains are not expulsive. Nor do they seem to move backward from the part in which they are felt, for no sound is audible over the cæcum either during the pain in the hepatic flexure or after it has disappeared. The contractions apparently occur again and again in the same region without moving in either direction. I have observed [1] in the cat such repeated circular contractions of the proximal colon, but they are not usual.

The recurrent pains ordinarily last from 6 to 8 seconds, increasing gradually in intensity until just before the end. They are commonly attended by gurgling noises audible as the cramp is passing away. The cramps have been observed succeeding one another for nearly

[1] CANNON: This journal, 1902, vi, p. 267.

ten minutes at intervals varying between 19 and 22 seconds, but in my experience they are ordinarily not so regular as this. The following figures, representing in seconds the time between the onset of successive cramps, illustrate the usual rather irregular recurrence of the contractions:

28	39	22	43
47	35	15	42
35	15	25	40
32	15	50	43
23	18	40	54
41	35	25	37

From the evidence I have been able to secure by auscultation and from sensations of cramp, it seems certain that the ascending and first part of the transverse colon are more active than the remainder of the large intestine. That there is an antiperistalsis in this more active region is not yet established. As already mentioned (p. 349), Elliott and Barclay-Smith have found that such sacculation as occurs in the human colon is associated with emphasized churning activity of the walls of the sacculi. In repeating their observations on the guinea-pig and rabbit I have seen oscillating movements of single sacculi, now here, now there, or of many sacculi at the same time, each contracting repeatedly, squeezing out the contents of the pouch, crowding full the neighboring pouches which in turn become active, then relaxing, filling, and discharging, again and again, till the food is thoroughly churned. Such a process could not be attended by a clearly marked rhythm, — too many little activities are going on at the same time. But these little activities would naturally be attended by the continuous popping noises and the slight gurglings which are heard at times over the ascending colon. Is it not likely that in man oscillating contractions of the walls of the sacculi, rather than antiperistalsis, is the prominent operation of the proximal large intestine? In accord with this suggestion is the observation above mentioned that the pains in the colon occur repeatedly in the same region, and do not spread to neighboring parts.

A characteristic sound, not periodic, which is audible at times along the transverse and descending colon is a progression of little crackling noises like the breaking of minute bubbles. The sound starts in the transverse colon, and its advance can be clearly traced. If the disc of the stethoscope lies over the splenic flexure, the crack-

ling can be heard first faintly, then louder and louder, then gradually more faintly again; and if after the climax of intensity there the stethoscope is changed to a position farther along the large intestine, the sound can again be heard passing through the same phases as before. This sound is likely to be followed immediately by a tendency to pass gas from the bowel.

Other Observations.

To one listening for the first time for rhythmic abdominal sounds, probably the most striking feature of what he hears is the large number of sounds which are not rhythmic. Most prominent among these irregular sounds are the sudden quick discharges or pops, which can be heard, either singly or in a short series of three or four, almost at all times and in all parts of the abdomen, though most frequently on the right side. As already stated, these reports resemble the sound of bursting bubbles; and it may be that they are caused by the squeezing of gas from a mass of the food by general pressure of the intestinal wall. Occasionally a continuous little gurgling can be heard for some moments, gradually becoming less intense. It seems probable that peristalsis in the small intestine is thus manifested.

A noteworthy characteristic of the intestinal sounds is their alteration in intensity and frequency at different times. I have no records showing this variation, but it has impressed itself upon me while listening for long periods to the activities of the intestines. At times there will be almost silence in the lower abdomen; the silence will give way gradually to an abundance of sounds, and these in turn will subside till again only occasional sounds are audible. Recent observations by Boldireff have proved that the alimentary canal has a periodic activity while not digesting;[1] the intestines may also have alternating periods of increased and decreased activity while digestion is going on.

Whether the observation of the sounds of the stomach and intestines is to be of clinical importance, will depend on whether there are typical variations of these sounds in different diseases of the alimentary canal. The observations here recorded, made chiefly upon myself, have been confirmed on other normal individuals. No attempt has been made to study the sounds produced in abnormal conditions. It has occurred to me that the method might be used to

[1] Boldireff: Archives des sciences biologiques, 1905, xi, p. 1.

separate the somewhat vague expression "motor insufficiency" into its two factors, absence of peristalsis and pyloric obstruction. Evidently if sounds recur in regular rhythm at the pylorus, and food remains in the stomach, the so-called "motor insufficiency" is due, not to absence of peristalsis, but to difficulty at the pylorus. This is only one of the applications which the method may have. In such disorders as gastritis, nervous dyspepsia, atony, colic, peritonitis, and dysentery, a study of the sounds produced by the movements of the alimentary canal, both before and after the administration of drugs, may reveal facts important to the clinician.

THE PERMEABILITY OF THE MEMBRANE OF THE EGG OF FUNDULUS HETEROCLITUS.

By ORVILLE HARRY BROWN.

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MEMBRANES of animal and plant cells vary in their permeability to salts and water. Those which allow the solvent, which is usually water, to pass through freely, and do not allow the dissolved substances to pass at all, — the so-called semi-permeable membranes, — are seldom or not at all found in nature. Most cell membranes are permeable to both water and salts, but usually the more permeable to the former. The membrane of the red blood corpuscle is perhaps the best example of a semi-permeable membrane. In such a cell osmotic changes occur if the isotonicity of the solution surrounding the cell is altered. But in case a cell has a membrane which is impermeable to both the solvent and the dissolved substance no osmotic tension can be developed. Or in case the membrane is equally permeable to the solvent and the dissolved substance, — the permeation occurring with ease or difficulty, — the osmotic tension is not developed when the molecular concentration of the liquid surrounding the cell is altered. The rate of diffusion through such a membrane is in direct proportion to the ease with which the molecules and ions permeate the membrane.

Fishes and other animals which are accustomed to living in the sea water usually die very soon after being placed in fresh or distilled water. It has been found by Bottazzi,[1] Fredericq,[2] Garrey,[3] and others that the blood or body fluid of marine invertebrates has the same freezing-point as the sea water from which the animal is taken, and therefore has the same osmotic pressure. A dilution or concentra-

[1] BOTTAZZI: Archives italiennes de biologie, 1897, xxviii, p. 61.

[2] FREDERICQ: Archives de biologie, 1904, xx, p. 709.

[3] GARREY: Biological bulletin, 1905, viii, p. 257.

tion of the aquarium water in which invertebrates are kept causes an equivalent change in the osmotic pressure of the body fluids. From this it is concluded that the membranes of the invertebrates are entirely permeable. The selachian blood is of the same molecular concentration as the sea water in which it lives. A dilution or concentration of the aquarium water in which it is kept causes a similar change in the body fluids, but death ensues before osmotic equilibrium is established. The membranes of selachians are evidently semi-permeable. The osmotic pressure of teleost blood is about half that of the sea water. This fact indicates that the fish either possesses some regulative mechanism which keeps the osmotic pressure of the blood constant or that the membranes are impermeable.

Garrey[1] made extensive abrasions of the skins of Fundulus heteroclitus, and placed them in fresh water, sea water diluted one-half, and sea water. Those in sea water diluted a half, which had the osmotic pressure of their blood usually survived, while practically all in the fresh water and normal sea water died. This seems to show quite conclusively that the Fundulus is resistant to changes in the salt concentration of the media in which it lives, because its membranes are impermeable to salts and water.

The young fish of Fundulus heteroclitus is even more resistant than the adult to changes in osmotic pressure. The eggs after being fertilized, as has been mentioned by Loeb[2] and others, develop equally well in sea, concentrated sea, fresh, or distilled water. Two explanations of this extraordinary phenomenon are apparent. The salts of the egg are lost when it is placed in distilled or diluted sea water, or increased when it is placed in concentrated sea water, and the protoplasm of the egg is able to develop without the salt or with more than the normal amount present; or the salt concentration of the egg is not altered when changes in the surrounding media are brought about.

Regarding the first possibility, it seems very unlikely from what we know of the importance of salts in protoplasmic activities that the egg could segment and form an apparently normal embryo, if practically all the salts are diffused out when the egg has been placed in distilled water. It likewise seems peculiar that this membrane should be of such a nature that water passes through it no more readily than the salts. This is evidenced by the fact that no change in the size of the egg is produced by altering the salt concentration

[1] Garrey: Biological bulletin, 1905, viii, p. 257.

[2] Loeb: Archiv für die gesammte physiologie, 1894, lv, p. 390.

of the media surrounding the egg. This was shown by Dr. Sollmann.[1] The membrane then must be equally permeable to salts and water, or semi-permeable with the power of resisting the force of osmotic pressure. This last, however, is inconceivable. The question of the permeability of the membrane of the Fundulus eggs is of much import on account of the large amount of work which has been done upon the egg.

In a former paper the author[2] of this article found that the Fundulus eggs were very difficult to polarize with an electrical current. The explanation offered for this was that the membranes were freely and equally permeable to water and ions. This explanation, however, is incorrect, as can clearly be demonstrated experimentally.

The eggs, when pinched between the points of a forceps, are found to be quite firm and resistant, somewhat resembling miniature golf balls. If they are removed from sea water and dried with filter paper, they withstand the effects of the room temperature for two hours before showing effects of the drying. If the eggs were freely permeable, it would seem that evaporation at a temperature ranging around 25° C. would take place more rapidly.

The eggs are not as good conductors as sea water. This is shown in the following way. A U-tube filled with sea water is balanced by a Wheatstone bridge against a resistance. The same tube is then filled with eggs displacing much of the sea water between the electrodes, and is again balanced against the same resistance. The slider has changed from 60.5 in the first case to 45 in the second, showing the resistance introduced by the eggs. When the sea water was poured out of the tube, leaving only the eggs extending from electrode to electrode and some sea water which remained on account of the capillary attraction, the resistance was further increased from 45 to 25.

To compare the eggs with non-conductors, the following experiment was performed. The tube containing eggs and sea water, as before, was balanced against a resistance. The eggs were then removed and small beads were substituted. The beads used were some larger than the eggs. The conductivity of the eggs was better than that of the beads, — the reading changing from 45 to 42½. There is really more difference between the two than this represents, because the beads, being larger than the eggs, did not fit together so closely as the eggs, and consequently more sea water was present.

[1] SOLLMANN: This journal, 1904, xii, p. 99.

[2] BROWN: This journal, 1903, ix, p. 111.

To ascertain when the salts of the eggs pass out, when the eggs are placed in distilled water, an experiment was performed as follows:

TABLE I.

Reading at 1.20 P. M. July 19 was	45.0	Reading at 4.00 P. M. July 20 was	74.0
" " 3.00 " " "	45.0	" " 8.00 " " "	76.5
" " 4.00 " " "	45.0	" " 10.00 A. M. July 21 was	76.5
" " 5.25 " " "	45.0	" " 4.00 P. M. " "	78.0
" " 9.30 " " "	45.5	" " 8.00 A. M. July 22 was	78.0
" " 9.00 A. M. July 20 was	51.0		

The eggs, which were obtained fresh, were washed rapidly through several changes of distilled water and placed in the U-tube along with some distilled water. The tube was then balanced against a resistance. Observations were then made at irregular intervals for a period of about seventy-two hours. Table I shows the records. During the first eight hours the conductivity remained practically unchanged, showing that no salts left the eggs. After the eggs had been in the distilled water eight hours, salts began to leave the eggs slowly, causing an increase in conductivity. After eighteen hours there was a period when the conductivity increased very considerably. After twenty-seven hours there was a slight increase in conductivity. To show that the salts are present in the eggs in an ionic condition before they begin to leave the eggs, the eggs were placed in distilled water in the U-tube as before and balanced against a resistance. They were then poured into a mortar and pulverized. The pulverized mixture was put into the tube and balanced against the same resistance. The Wheatstone bridge showed that the increased conductivity was from 70 to 90.

Mathews[1] found that after the eggs had been in distilled water for some hours considerable chlorine was still present in the eggs.

An experiment was tried to demonstrate that salts pass into the eggs. The eggs were placed in concentrated sea water in the U-tube and balanced against a resistance. If salts entered the egg and water left it, there should be a decrease in conductivity of the tube. But there was invariably a slight increase in conductivity instead of a decrease. This was due to fermentative changes which took place in the eggs after they had been in the abnormal conditions for some hours. This usually occurred after the eggs had been in the concentrated sea water ten to twenty hours. Since it was about at this time

[1] MATHEWS: This journal, 1904, x, p. 290.

that salts seemed to leave the eggs most plentifully when they were in distilled water, the possibility is suggested that the increased permeability of the membrane may be due to a fermentative process. That salts do pass into the eggs is shown by Mathews'[1] and others' experiments where toxic effects are obtained by surrounding the eggs with solutions of the toxic agent. Stoccard[2] found, by placing the eggs in a lithium solution, that if the eggs were removed in three, four, or five hours, no toxic effects were produced; but after that time characteristic effects resulted.

Conclusions.

The membrane of the eggs of Fundulus heteroclitus is practically impermeable to salts and water during the first six or eight hours, *i. e.* eggs placed in distilled water do not lose their salts during that period. The membrane becomes the most permeable after about eighteen or twenty hours. The membrane is equally permeable to water and salts.

My thanks are due Dr. Carlson for helpful suggestions.

[1] Mathews: This journal, 1904, x, p. 290.
[2] Stoccard: Personal communication.

ON THE SUPPOSED EQUIVALENCE OF SODIUM AND LITHIUM IONS IN SKELETAL MUSCLE.

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THE importance of electrolytes in suitable variety and in balanced relations as a condition of normal activity in living tissue has in recent years constantly gained recognition. At least four metallic ions seem always to be present in measurable quantity in animal cells and fluids. These are Na, K, Ca, and Mg. The action of Ca and K has been defined through many experiments — exact studies in regard to Mg are wanting. Among these ions Na occupies a unique and baffling position. Its great abundance in the fluids precludes the attempt to find out its specific influence by increasing the proportions present — for to increase its quantity is to make the solution hyperisotonic and so to introduce disturbing conditions for which we can scarcely make due allowance. To reduce the sodium in our media, seeking to discover in what ways the tissue suffers by its withdrawal, is likewise an unsatisfactory process, for we must either permit a damaging dilution or we must substitute for the normal Na an isotonic equivalent of some foreign solute which we cannot positively know to be indifferent to the protoplasm.

However, in spite of these difficulties, some little evidence in regard to the importance of sodium has accumulated. It has become clear through the studies of Loeb,[1] Lingle,[2] and especially of Overton,[3] that the ion is not merely useful in maintaining osmotic relations, but has a more intimate function. For instance, Overton has shown that while the contractility of a muscle is soon suppressed by immersion in cane-sugar solutions, a very little sodium, one-tenth of the normal, in such a sugar solution is sufficient to keep the immersed muscle responsive to stimulation.

[1] Loeb: Fick's Festschrift, Braunschweig, 1899, p. 99.

[2] Lingle: This journal, 1900, iv, p. 265; 1902, viii, p. 75.

[3] Overton: Archiv für die gesammte Physiologie, 1902, xcii, p. 346.

Overton was led to believe that sodium has an essential part in the excitation process within the muscle and one that is simulated by a single foreign ion, namely, Li. In the range of his observation lithium chloride seemed to be quite equivalent to sodium chloride. The harmlessness of lithium salts had been remarked in a casual way by Loeb some time before. Both Loeb and Overton have called attention to the fact that these two ions have about the same migration velocity. It seemed desirable to make a further comparison between sodium and lithium to ascertain whether the latter does not in some respects come short of the native sodium, as affecting the performance and length of life of skeletal muscle.

The two ions are not equivalent for the maintenance of spontaneous rhythm in cardiac tissue. Lingle has shown this conclusively. Still, the maintenance of automatic contractions is a different matter from the preservation of irritability, and it was this criterion to which we looked in our study of striated muscle. Lingle had found — as had Howell[1] — that while lithium cannot be substituted for sodium to the exclusion of the latter if the heart is to beat for a long time in the mixture, a large part of the sodium may be successfully replaced, and further, that a protracted immersion of the heart strip in lithium chloride indeed keeps it quiescent, but does not prevent a return of activity when a normal medium takes the place of the lithium solution. In other words, it is quite possible that a lithium solution may keep cardiac cells in a state of inhibited activity without injuring them.

In each of our experiments two companion muscles of the frog were taken for comparison. They were immersed for the same period in different solutions, and when subjected to stimulation they were made to form links in a common circuit for the passage of the current derived from the induction-coil. It was our intention that the stimulation should be supra-maximal, but this condition was not always attained. A mechanical device was employed for eliminating make-shocks, and the break-shocks were sent in by means of a cog-wheel interrupter giving about thirty stimuli per minute. In this way we recorded series of contractions from both muscles at once, and were thus enabled to judge not only the comparative magnitude of the responses immediately after bathing in various solutions but the respective endurance of the two muscles. This made our examination a much more searching one than it would have been had we

[1] HOWELL: This journal, 1901, vi, p. 181.

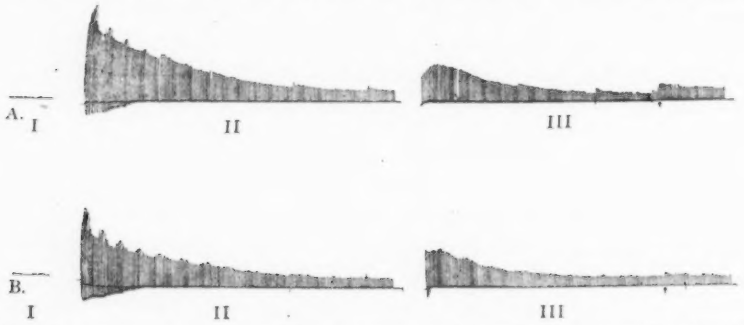
been content with obtaining a few contractions. The series often comprised 600 twitches or more, and sometimes, when the two muscles at first gave an equal performance, one kept up better than the other, and significantly excelled it in the aggregate of work done. Bathing and stimulation were repeated until one or both muscles had practically ceased to act.

The solutions we first compared were a standard Ringer mixture for the control, and what may conveniently be called a lithium Ringer, lithium chloride being substituted for the sodium chloride of the normal fluid. Freezing-point determinations were made and the concentration of the two corresponded closely ($-0.50°$ and $-0.54°$). In each the amount of potassium chloride was 0.03 per cent and of calcium chloride 0.025 per cent.

The muscles used were the gastrocnemius and the sartorius. Each has its advantages, — the first in its exemption from injury in dissection, and the second in its thinness, which must be favorable to a thorough penetration by the salts. The two always yielded concurrent results.

How free is the exchange of ions between the immersed muscle and the medium? The uncertainty upon this point is the chief obstacle in all such studies. We were led constantly to more and more protracted experiments, seeking to have the substitution as complete as possible, and we could never escape doubts as to our success. Overton has given reasons for believing that the ions do not pass freely through all parts of the muscle-substance, but that their movements are governed by temporary local conditions, such as the phases of the contraction process. If the activity of the muscle promotes the diffusion of the ions within it, the procedure in our experiments was highly favorable to such migration, for the practice of alternate bathing and stimulation should provide the desired conditions. Another consideration tends to increase our confidence in the method. We readily confirmed Overton's observation that an isotonic sugar-solution speedily abolishes irritability, and we accept his inference that this is due to the withdrawal of sodium. Now, if the exposure to sugar has not been too long, we can restore the muscle quite successfully by returning it to ordinary Ringer or fully as well by placing it in "lithium Ringer." In the latter case we feel sure that the native sodium has been very largely replaced by the lithium. Such an experiment needs only thirty or forty minutes' bath in sugar and an equal time in the restorative.

Other salts than lithium chloride seem to penetrate the muscle so rapidly that we are inclined to think that the difficulty is less than has been assumed. Certain experiments which we made aside from the main course of our work emphasized this. We compared not only "sodium Ringer" with "lithium Ringer," but we also made up mixtures with similar potassium and calcium content in which the place of the sodium chloride was taken by ammonium chloride and by magnesium chloride. It is probable that all other chlorides are more



Two muscles were kept thirty minutes in a solution of dextrose plus potassium chloride and calcium chloride (percentages as in Ringer). (I) shows the practical failure of both muscles to contract when stimulated, — a failure which Overton has shown not to be due to the increased electrical resistance of the tissue. *A* was then bathed twenty minutes in "lithium Ringer" and *B* in "sodium Ringer." The second period of stimulation (II) shows a recovery which is quite as marked in *A* as in *B*. After thirty minutes longer immersion, *A* being still in "lithium Ringer" and *B* in the normal, a third period of stimulation again shows the essential equality of condition in the two muscles.

decidedly toxic than these, yet a muscle kept for an hour in "magnesium Ringer" is found to have failed greatly as compared with the control in regular "sodium Ringer." This is more strikingly true of a muscle in "ammonium Ringer." The injury which these muscles betray must be due to the entrance of the foreign ions, the loss of Na-ions, or both. But if the exchange takes place so easily in these cases, may we not assume that Li-ions really replace Na-ions in our prolonged experiments with the bath of "lithium Ringer"?

We may now pass to a discussion of our results and of what they teach concerning the equivalence of sodium and lithium. As long as our experiments did not last more than a few hours, the records obtained from muscles in lithium solutions were quite equal to those of the controls. A superficial view of the tracings indicated that it

was immaterial whether Na- or Li-ions were offered, and Overton's claim seemed fully confirmed. But we found, even in these shorter experiments, that the performance of the two muscles was equal only when the stimuli applied were distinctly excessive. With moderate stimulation the normal muscle would do far better work than its fellow in "lithium Ringer." The substitution of lithium for sodium in the muscle has not been without a depressing influence upon its irritability.

Longer experiments were resorted to. Pairs of muscles were kept in the ice-chest for one, two, and three days. When set up and stimulated they showed much more clearly the same fact, — that lithium makes a muscle difficult to excite, but that a strong stimulus may still produce contractions which are nearly as high as can be evoked from the control.

Finally, we made the following trial. Two muscles had been treated as just described; after seventy hours the one in normal Ringer was in good condition, while its companion was in a state of low irritability. This muscle was transferred to "sodium Ringer," and after three hours more a concluding trial showed that the Li-muscle had substantially recovered its excitability, and in its last series of contractions nearly equalled its mate, which had been all the time in the normal solution.

The evidence seems to warrant the conclusion that the chemically similar ions, Na and Li, are closely related physiologically but are not equivalent. There is no foreign ion which is so slow to make a noteworthy change in the properties of skeletal muscle as Li, but when much of the sodium is at last replaced there follows a characteristic fall in irritability. On the other hand, no irreparable harm seems to result; we may restore the muscle by a reversal of treatment, returning Na-ions in place of the Li-ions previously introduced.

If we are right when we infer that the specific effect of lithium consists largely at least in a blunting of excitability without entailing any corresponding loss of latent contractility, we can harmonize the phenomena seen in the skeletal muscle with those exhibited by the heart. The cardiac cells receiving Li-ions and parting with Na-ions may suffer the same loss of irritability, and become quiescent because the internal stimulus is no longer adequate. Yet the subsequent removal of the lithium may leave the tissue in excellent condition, with its store of energy as available as before.

While a distinct difference between lithium and sodium can thus be shown to exist, it remains true that the correspondence between the two is striking. There is perhaps only one other case in which a kation native to the tissues can be replaced with any degree of success by another member of the chemical group to which the metal belongs. This is the substitution of strontium for calcium which Ringer[1] found to be possible. Various attempts have been made to account for the divergent behavior of elements nearly related chemically by connecting their physiological properties with sundry physico-chemical characters, — atomic weight, valence, ionic velocity, decomposition tension, etc. Desirable as it is to systematize the properties, we do not feel that progress in that line has yet been very satisfying. Between Na- and Li-ions the physiological differences are not great. Compared with the contrasts existing between Na- and K-ions, they are minimal. Where the physical chemist finds moderate differences of property the physiologist finds immeasurable differences. The findings of the chemist are numerical and comparable, while those of the physiologist are often qualitative and incommensurate.

It should be noted that the harmlessness of lithium chloride as demonstrated for skeletal muscle does not obtain for all tissues. It is said to be quite poisonous to the alimentary tract,[2] and its action upon nervous structures is probably far from that of sodium chloride. More work upon this question is to be hoped for.

It does not seem profitable to compare our results with those obtained from studies of cilia, eggs, and larvæ. Such forms of protoplasm as are normally in direct contact with a fortuitous and varying medium show in remarkable degree a tolerance for many salts. How far this is due to powers of selective absorption and exclusion cannot be estimated. Certainly the essentially internal cells of the higher animals, with their natural environment of a nearly constant character, are far more sensitive to violations of composition in the surrounding fluid.

While we pursued our chief object a secondary question suggested itself. If the Li-ion lacks so little of being a perfect substitute for the Na-ion, may we not hope that the addition of a small amount of some other salt may give a mixture still more closely approximating the normal fluid? Our attempts to discover such a mixture have not

[1] RINGER: Practitioner, 1883, p. 117.

[2] CUSHNY: Pharmacology, Third Edition, 1903, p. 499.

yet been numerous enough to justify positive assertions. But we have been impressed by the favorable character of a solution in which the normal sodium chloride is replaced by the chlorides of lithium and magnesium in the proportion of four to one. As has been stated, magnesium chloride alone is distinctly unfavorable, but when it is present with an excess of lithium chloride no trace of its injurious effect remains noticeable. On the contrary, muscles kept in this compound mixture gave us exceptional records, and on the whole surpassed the controls in "lithium Ringer." But the straight lithium mixture is so good a medium that it is hard to demonstrate that the other is really an improvement. If we are confirmed in our present impression, an interesting example of antitoxic action of salts will be added to the list made known by Loeb,[1] Mathews,[2] and other workers in this field.

[1] LOEB: This journal, 1902, vi, p. 411.

[2] MATHEWS: This journal, 1905, xii, p. 419.

PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES OF MAGNESIUM SALTS. — I. GENERAL ANÆSTHESIA BY SUBCUTANEOUS INJECTIONS.

By S. J. MELTZER and JOHN AUER.

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Introduction.

THE salts of sodium, potassium, calcium, and magnesium are the chief inorganic compounds present in the tissues and fluids of the animal body. As to the rôle which these elements might play in the manifestations of life phenomena, the first three have been the subject of a vast number of studies in experimental physiology; magnesium, however, received hardly any attention. Wherever calcium is found in the body magnesium is also present. Recently calcium has received considerable attention. While there is as yet no unanimity of opinion concerning the actual part which calcium takes in the activities of the living body, all agree that it is an important factor in the mechanisms which are at the bottom of these activities. Should magnesium be no active factor in these mechanisms? Should magnesium, for instance, have no active share in the irritability of nerve and muscle tissues? This would be the more surprising since in muscle and nerve tissues magnesium is present in larger proportion than calcium, and it therefore seems that the affinity of those tissues is greater for magnesium than for calcium. This view is strengthened by feeding experiments, at least as far as muscle is concerned. When animals are fed magnesium compounds, that element is found greatly increased in the ash of muscles, while the percentage of magnesium in bone is but slightly greater than under a normal diet.[1] As nerve cells, nerve fibres, and muscle fibres are the most irritable, the most active tissues of the body, we should expect magnesium to take some essential part in the activities of the body, at least in the specific activities of the tissues mentioned.

[1] König: Zeitschrift für Biologie, 1874, x, p. 69.

However, the share which magnesium might be taking in the specific manifestations of nerve and muscle tissues need not be of the character which increases its specific activities. It is possible that the presence of magnesium is not at all for the purpose of increasing the specific energies of muscle and nerve, of favoring the contractility of muscle fibres, or of facilitating the conductivity of the nerve fibres, or the receptability and conductivity of nerve cells; on the contrary, the task of magnesium might be to render assistance in restricting, reducing, or inhibiting all the specific energies of these tissues. At a certain phase of functional activity the inhibition of a contraction of a muscle, or the inhibition of a reflex action, or of a sensation within a ganglion cell, is an indispensable factor in the prompt working of these functions, and these inhibitions require as much vital assistance for their development as is required and received by the positive energies of these tissues. The diastolic part in the cycle of a heart beat; the inhibition of the contraction of the inspiratory muscles in the cycle of a respiration; the inhibition of the disturbing activity of any antagonistic muscle; the inhibition of a disturbing concomitant reflex; the inhibition of the activity of one sense during the act of perception of another sense, — in these and in many other instances the suppression, the inhibition of an activity is an indispensable part of a vital function. Is it not possible that the processes of inhibition are essentially facilitated by compounds which contain magnesium?

This supposition suggested itself to us by an observation which one of us (M.) made a few years ago. In studying the effect of intracerebral injections it was found that magnesium sulphate acted differently from some of the other inorganic salts. In a paper[1] read at the meeting of the American Physiological Society in December, 1899, the effect of an intracerebral injection of potassium chlorate was described as producing a long series of convulsions, forced movements, opisthotonus, etc. The reported proceedings of this meeting contain the following passage: "The author demonstrated on a rabbit the described effects of potassium chlorate, and also on another rabbit, the *opposite effect of intracerebral injection of magnesium sulphate: without preceding convulsions the rabbit became paralyzed in a short time.*" From this observation it seemed that the primary effect of magnesium upon nerve cells is that of paralysis without any preceding excitation. At that time no comment was made by the author

[1] MELTZER: This journal, 1900, iii, p. ix.

upon the demonstrated phenomenon; it was reserved for a further experimental analysis.

In the past year a number of experimental studies were made by us on the effect of magnesium salts upon the activity and irritability of the nervous system. The studies brought out some new facts which will be reported in several communications. In the present communication we shall deal with some effects of subcutaneous injections of magnesium salts. We may state at the outset, in a general way, that the several series of experiments we have carried on so far with magnesium salts seem indeed to confirm our original expectations: 1. Magnesium exerts a profound effect upon the nervous system. 2. The effect seems to be exclusively of an inhibitory character; we had so far not a single instance of an increase of excitability brought on by a magnesium salt.

However, in describing our several results we shall lay more stress upon the facts which our investigations bring to light than upon any general theory which our experiments may tend to prove.

Subcutaneous Injections of Magnesium Salts.

As we have already stated above, and as the sub-title indicates, we shall report in the present communication the observations made in the series of experiments with subcutaneous injections of magnesium salts. Such experiments have been made before. They were undertaken, however, from another point of view, and the results which they brought out were different from those we are able to communicate. In the seventies of the last century many experiments were made with subcutaneous injections of small doses of magnesium sulphate. The purpose of these experiments was to verify the statements made by Luton [1] and by Vulpian that injections of small doses of that salt produce purgation. Recke [2] reported later that subcutaneous injections of large doses of magnesium sulphate can be fatal to the animal. The same was claimed by Hay,[3] who stated that death occurs by paralysis of respiration. Mickwitz [4] claimed that magnesium chloride stops the heart before it can affect the respiration. Clessin [5] also studied

[1] Luton: Gazette hébdomadaire de médecine et de chirurgie, 1874, xxviii, p. 455.

[2] Recke: Dissertation, Göttingen, 1881.

[3] Hay: Journal of anatomy and physiology, 1883, xvii, p. 512.

[4] Mickwitz: Dissertation, Dorpat, 1874.

[5] Clessin: Dissertation, Würzburg, 1890.

the toxic effect of chloride of magnesium upon the heart and muscles of the frog.

We employed in all of our experiments comparatively large doses of the salts, which gave indeed fatal results in some cases, and which we shall also describe. However, the effect which we have studied chiefly, and which was never studied or described before, is the production of anæsthesia by these salts. As our protocols will show, we obtained complete anæsthesia with subsequent full recovery in dogs, cats, rabbits, guinea-pigs, white rats, frogs, and fowls. For most of our experiments we have employed magnesium sulphate in 25 per cent solution. We have however established by a number of experiments that anæsthesia can also be produced promptly by magnesium chloride, and in a few tests we obtained similar results with magnesium bromide. We shall present our results by giving a few selected protocols from each series of experiments.

SUBCUTANEOUS INJECTIONS OF MAGNESIUM SULPHATE IN RABBITS.

Experiment 1. April 13. — Gray rabbit, female, 1500 gm., very active and restless; 120 respirations to the minute.

3.17 P.M. Injected subcutaneously into left upper flank 8 c.c. of a 25 per cent solution of magnesium sulphate.

3.20. Jumps off table, moving about floor in lively fashion, kept there.

3.30. Losing interest in surroundings.

3.40. Remains quietly in centre of room when put there; when forced to hop, hind legs are moved clumsily.

3.50. Practically unable to move; hind legs weaker than front legs.

3.51. Can be placed lying on side; front leg when placed behind ear remains there. Limbs perfectly flaccid, ears on back, chin on table. Limp as a rag when picked up. Probe in nasal cavity provokes only moderate resistance; pinching tail arouses animal somewhat.

3.59. 68 respirations to the minute.

4.00. Conjunctival reflex abolished; is not aroused by a noise close to the ears, but withdraws head slightly when probe is inserted into nose. Remains in practically the same condition for about two hours.

6.00. Animal recovered.

April 14. — Animal lively as ever; had no diarrhœa.

This animal received a subcutaneous injection of magnesium sulphate, 1.3 gm. per kilo, and was not massaged. About forty minutes after the injection the rabbit was apparently in a state of

anæsthesia; every muscle was perfectly relaxed and the conjunctival reflex was gone. After remaining in that state for about two hours it recovered completely.

Experiment 2. May 2. — Gray rabbit, male (201), 1640 gm.; 64 respirations to the minute.

3.30 P.M. Received a subcutaneous injection of 10 c.c. of a 25 per cent solution of magnesium sulphate in right back. About 1 c.c. escaped. Place massaged.

4.05. Lying on side, unable to get up, front leg kept behind ear, conjunctival reflex very sluggish.

4.12. Held up by legs, no struggle, limp as a rag; no conjunctival reflex; no response to pinching tail.

4.30. Respiration 64 to the minute. Condition unchanged.

5.00. Got on his feet.

May 3. — Animal very active.

This animal received perhaps a little more magnesium sulphate than the previous one, and the place of injection was massaged. The anæsthesia, which also set in about forty minutes after injection, reached here a complete stage, but lasted hardly an hour.

Experiment 3. May 3. — White rabbit, female, 1420 gm.; 200 respirations to the minute.

4.07. Injected subcutaneously into right flank 12 c.c. of a 25 per cent solution of magnesium sulphate; massaged.

4.16. Animal sits quietly, weak and shaky when moving.

4.20. Lying motionless on side, breathing slow and very shallow.

4.22. Pupils dilated. Often weak kicks of hind legs, perfunctory convulsions from asphyxia; artificial respiration instituted.

4 26. Heart stopped beating.

This animal received 2 gm. per kilo, and the place of injection was massaged. Muscular weakness developed very rapidly, the respiration became very shallow, and fifteen minutes after injection complete asphyxia set in, but with only very slight indications of convulsions.

Experiment 4. May 4. — Gray rabbit, female, 1430 gm. (204, survivor from a previous subcutaneous injection). 76 respirations to the minute.

3.53. Injected subcutaneously into left flank 9 c.c. of a 25 per cent solution of magnesium sulphate; massaged.

4.06. Just about able to move; 128 respirations to the minute.

4.15. Lies flat on side as placed.

4.37. No conjunctival reflex; animal limp as a rag, perfectly flaccid.

5.12. 52 respirations to the minute; inspiratory pause and active expiration.

6.05. Still flaccid, occasional slight movements of legs.

6.15. Got feet under body. Observation discontinued. Put into cage.

May 5. — Animal normal, had no diarrhœa.

This animal received 1.5 gm. magnesium sulphate per kilo, and was massaged. The anæsthesia was most complete; it set in early and lasted over two hours. The rabbit survived two injections without any after effect.

Experiment 5. — Gray rabbit, male, 1700 gm.; 160 respirations to the minute.

2.53. Injected subcutaneously into right flank 12 c.c. of a 25 per cent solution of magnesium sulphate.

3.22. Animal lying limp on back, no resistance when raised by leg.

Animal operated without any other anæsthesia. Cannulæ introduced into trachea, left carotid artery, and left pleural cavity; left vagus cut. Operations completed at 4.04. There was no struggle whatsoever, and no sign of pain throughout the operation.

Until 5.30 tracings of blood pressure and respiration were taken, and the central and peripheral ends of vagus often stimulated with induction currents (see Figs. 1 and 2). The blood pressure taken about 75 minutes after the injection of the magnesium salt amounted to nearly 100 mm mercury; one hour later it fell to about 75 mm. Stimulation of the central end of the left vagus, which produced a strong effect upon the respiration, brought out no sign of pain. The blood in the carotid artery looked dark, although the respiration was good.

5.30. Trachea clamped: asphyxia brought out convulsions. Autopsy. Lively peristalsis of small intestines, which contain liquid material and gas. Bladder contains 30 c.c. of urine, which shows a powerful reduction with Trommer's test for sugar; and with sodium hydrate and Lugol's solution a strong odor of iodoform was produced (aceton).

In this experiment the animal received 1.75 gm. magnesium sulphate per kilo. It had a most perfect narcosis in about thirty minutes. A very extensive operation and usually painful stimulations could be carried on for nearly two hours without any sign of a pain reaction. Nevertheless the blood pressure, at least for the first part of the experiment, was nearly normal. Judging from the effect of stimulation of the central end of the vagus upon the respiration, the medulla oblongata was apparently still very sensitive. Had we in-

terrupted the experiment and sewed up the wound, the animal probably would have survived the injection, operation, and experiment,

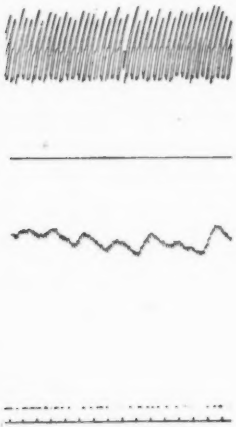


FIGURE 1. — One-half the original size. Blood pressure and respiration tracing from a rabbit (Experiment 5) seventy-five minutes after receiving a subcutaneous injection of magnesium sulphate, 1.75 gm. per kilo. Blood pressure was obtained with a mercury manometer, the fluid within the connecting tube being a mixture of sodium carbonate with hydrated sodium carbonate. The respiration was written by means of Meltzer's pleural cannula (see Zeitschrift für Instrumentenkunde, December, 1894). The upper tracing represents the respiration, upstroke the inspiration; the straight line below it marks the atmospheric pressure, and the space between corresponds to the negative pressure during expiration. The next wavy tracing represents blood pressure; the straight line below it is the 0-line for blood pressure. The lowest line marks the time in two-second intervals for both tracings, which were taken simultaneously as they are presented in this figure.

FIGURE 2. — One-half the original size. From the same animal as in Fig. 1, taken sixty minutes later. Tracings and lines the same as in Fig. 1, with a slower drum. The respiration curve shows the effect of stimulations with induction currents of the central end of the cut left vagus (250 and 200 mm. distances between the coils). The blood pressure curve shows the effect of a stimulation (200 mm. distance) of the peripheral cut end of the vagus upon the heart. The blood in the cannula began to clot, hence the small excursions.

regaining his normal state, — an occurrence we actually observed in other somewhat similar experiments.

These few abbreviated protocols illustrate sufficiently the essential results which we obtained in the series of experiments with subcuta-

neous injections of magnesium sulphate in rabbits. With a certain dose of this salt we could obtain a complete anæsthesia lasting as long as two hours and even longer, from which the animals recovered without any perceptible after-effect. During this state of anæsthesia any operation could be performed and nerves stimulated without causing any pain or waking the animal. The muscles are completely relaxed. We may add here that direct and indirect stimulations of the muscles brought out prompt reactions. In some experiments the abdomen was opened during the anæsthesia without there being the slightest attempt to press the intestines out of the cavity. The conjunctival reflex was completely abolished; a probe in the nose would be tolerated without any reaction. (In this connection we have, however, to mention an instructive observation. Even when the animal was under such deep anæsthesia as not to react to the presence of a probe in the nose, the application of ether to the nose would stop the respiration immediately, producing passive expiration, or, in other words, *ether would inhibit the inspiratory contractions.*) The respirations were usually reduced in number. The blood pressure was not much below the normal.

The dose of magnesium sulphate which causes complete anæsthesia lies between 1.75 and 1.25 gm. per kilo for the rabbit. A dose above 1.75 per kilo is usually fatal for the animal, and if the place of injection is massaged the fatal issue develops quite rapidly. The animal becomes paralyzed within a few minutes, and respiration ceases before the heart stops; at this stage artificial respiration does not save the animal, as the large dose of magnesium apparently affects also the blood pressure and heart beats, though in a lesser degree. We have lost two animals with only 1.75 gm. per kilo after an energetic massage of the injected area.

A dose of 1.25 per kilo is about the lower limit. Sometimes even a smaller dose will produce an apparently satisfactory anæsthesia and permit an operation, but then stimulation of a sensory nerve would rouse the animal.

The motor impairment seems to precede the anæsthetic influence; when small doses were given, the animal would be unwilling to move around, while the reflexes would still act perfectly.

No anæsthetic dose interfered with the reaction of the pupil to light. A fatal dose would soon cause a dilation of the pupil.

Intramuscular injections.—We did not make numerous intramuscular injections with any of the magnesium salts. We believe, however,

that we are justified in making the following brief general statement. The effect of an intramuscular injection of magnesium salts is somewhat superior to that of a subcutaneous one, but is by far inferior to the effect of an intravenous application.[1]

Furthermore, the intramuscular injection of these (concentrated) salt solutions seemed to favor incidentally the development of abscesses. Whereas in the subcutaneous injections in rabbits we never had an abscess, sloughing abscesses developed now and then after injections into the muscles. We have to add, however, that the solutions used were not sterilized.

Magnesium Chloride in Rabbits.

In order to prove that the anæsthetic effect which we obtained with subcutaneous injections of magnesium sulphate was due to the magnesium-ion of that salt, we have tested the effect of magnesium chloride after subcutaneous injection. In order to have about the same molecular concentration as the sulphate salt, we used a 19 per cent solution of magnesium chloride — about 2 M. The few experiments we have made, proved beyond doubt that magnesium chloride also exerts a profound anæsthetic effect after subcutaneous injection. With this salt we have not tried to reach the maximum limit; on the contrary, the doses we have used were rarely above 1.0 gm. per kilo, which is about equimolecular with 1.3 gm. of magnesium sulphate, — the latter dose being about the minimum limit for that salt. We will illustrate our experience with one protocol.

Experiment 6. May 9. — Gray rabbit, female, 1100 gm.; respirations about 90 to the minute.

3.40. Injected subcutaneously into right flank 5.5 c.c of a 19 per cent solution of magnesium chloride; massaged.

4.06. Animal unable to move; front leg placed behind ear, stays there.

Animal operated; no resistance and no sign of pain. Cannulæ into trachea, left carotid artery, and left pleural cavity. Left vagus cut. Blood in carotid artery bright red. Operation finished at 4.40. Graphic record of the blood pressure and respirations obtained, and central end of vagus stimulated. (See Fig. 3.)

5.15. Abdomen opened; animal shows no pain, no attempt to press out the intestines from the cavity. No peristalsis of small intestines,

[1] Meltzer and Auer: Journal of experimental medicine, 1905, vii, p. 59.

which contain liquid and gas. Sciatic nerve stimulated: good contraction of leg, but no pain. Blood still bright red. Animal killed. Bladder contains about 10 c.c. of urine; no reduction with Trommer's test.

This rabbit received only 1 gm. of magnesium chloride per kilo animal. About thirty minutes after injection the animal was operated, and nerves were stimulated without any struggle and without any sign of pain. At the same time respiration was good, blood bright red, and blood pressure nearly normal. No anæsthesia could be deeper and more harmless at the same time.

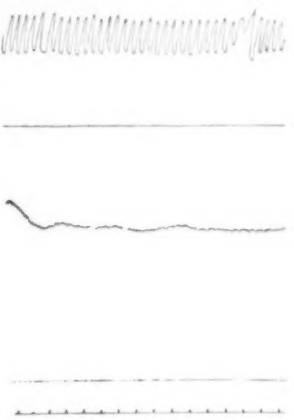


FIGURE 3. — One-half the original size. Tracings obtained from rabbit (Experiment 6) two hours after receiving a subcutaneous injection of 1.0 gm. magnesium chloride per kilo body weight. Tracings and lines the same as in Fig. 1.

SUBCUTANEOUS INJECTIONS OF MAGNESIUM SULPHATE IN CATS.

In a general way our experience with rabbits holds good for cats also. A certain dose of the salt will produce anæsthesia with recovery, while the anæsthesia produced by a larger dose terminates earlier or later in death of the animal. There are, however, some differences between the behavior of the two species towards the subcutaneous injection of magnesium salts. In the first place, the zone of the anæsthetizing non-fatal dose of the salt for the cat is very narrow. For instance, the anæsthesia brought on by a dose of 1.0 gm. per kilo cat terminates fatally, while that produced by 0.8 gm. per kilo was still unsatisfactory. Only a dose of 0.9 gm. of magnesium sulphate produced a satisfactory narcosis from which the animal recovered completely. This statement already contains the further point of difference that cats are very sensitive towards the toxic effect of magnesium salts. Furthermore, the fatal issue, which a larger dose brings on in cats, is mostly preceded by a long stage of deep anæsthesia, at the beginning of which the condition of the animal may be excellent; but the anæsthesia gradually deepens, the respiration becomes more and more slow and shallow; the animal dies without any sign of asphyctic convulsions. The anæsthesia in cats, as a rule,

sets in much later than in rabbits. The following few protocols will illustrate these statements:

Experiment 7. May 19. — Yellow cat, male, 3820 gm.

3.40 P. M. Injected subcutaneously into left flank 15 c.c. of a 25 per cent solution of magnesium sulphate.

3.45. Respirations 64 to the minute.

4.45. Respirations 128 to the minute, deeper than before (?), salivated, staggers in cage.

4.55. Lying on the floor on one side with relaxed extended extremities; conjunctival reflex present; stepping on tail causes no reaction.

5.25. Lying on the same spot and in the same manner as before; 40 respirations to the minute, moderately deep; conjunctival reflex present; stepping on tail — no reaction; on inserting probe in nose, moves head slightly.

6.00. Respirations 28 to the minute, fairly deep; otherwise no change.

6.22. No change. Observation discontinued.

Next day found dead in the same position as left on the previous evening.

This animal had only 1.0 gm. per kilo of the magnesium salt. In this animal, as well as in animals which received larger doses, the anæsthesia did not set in until about one hour after injection. Then the anæsthesia became profound, lasted long, and terminated finally in death. It is noteworthy that in cats the conjunctival reflex was never fully abolished even when the anæsthesia was so deep that no response was produced by stepping heavily on the tail, cutting the peritoneum, or stimulating sensory nerves.

Experiment 8. May 23. — Male cat, 3330 gm.

3.09 P. M. Injected subcutaneously into left side 10.6 c.c. of a 25 per cent solution of magnesium sulphate, moderately massaged.

4.45. Staggers slightly when walking, which is done in crouching fashion.

5.00. No change; but can be stretched out on board without struggling; not operated. Survived.

Experiment 9. May 26. — Same cat used in last experiment; weighs now 3050 gm. (Lost about 10 per cent of his weight in three days.)

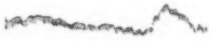
2.45 P. M. Injected subcutaneously in side 11 c.c. of a 25 per cent solution of magnesium sulphate.

3.15. Can still move, but with incoordination and marked weakness. Placed on back and nails clipped without resistance.

3.45. Tied on board, femoral artery exposed and cannula inserted, crural nerve handled, tail pinched: no sign of pain. Very slight movements of body now and then. Blood pressure about 125 mm. mercury (see Fig. 4).

4.05. Finished taking blood pressure tracing; wound sewed up; no sign of pain. Taken from board and placed on floor; staggered in crouching position to a corner.

May 27. Animal survived. No diarrhœa. Seems well. Did not lose weight within the next three days.



The animal used in the last two experiments was only moderately affected by a dose of 0.8 gm. per kilo (Experiment 8). But with a dose of 0.9 gm. per kilo (Experiment 9) the animal was in a satisfactory state of anæsthesia. It could be operated without any sign of pain. The blood pressure was fairly high, the blood bright red, and the animal survived without suffering any after effects.

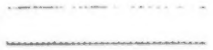


FIGURE 4. — One-half the original size. Blood pressure tracing from cat (Experiment 9), seventy minutes after receiving a subcutaneous injection of 0.9 gm. magnesium sulphate per kilo. Upper curve blood pressure, lower straight line 0-line, interrupted line time in two seconds.

It is noteworthy that in cats the anæsthesia set in before the abolition of all voluntary and reflex movements.

Some of the cats vomited at the onset of the anæsthesia. Salivation was not frequent. The urine of the cats which received magnesium sulphate reduced copper sulphate.

Magnesium chloride produced in cats about the same effect as the sulphate. In two young cats doses of 1.0 and 1.2 gm. per kilo produced only slight effects.

No abscesses were produced in cats by subcutaneous injections of the magnesium salts.

Injections of Magnesium Salts in Dogs.

These experiments have demonstrated that this species of animal can also be completely anæsthetized by injections of magnesium salts. In this series we had, however, to contend with some difficulties. In the first place we had to deal with large animals which, in consequence, required large quantities for the production of anæsthesia. In the second place the absorption from the

subcutaneous tissues of these animals seemed to be distinctly inferior to that of rabbits and cats. Even after considerable massage of the injected place a bulky mass would often remain for hours and even for days and would frequently lead to an extensive abscess. An injection of 1.5 gm. of magnesium sulphate per kilo never led to any satisfactory anæsthesia. A dose of 2 gm. per kilo or a little more would, after some time, anæsthetize the animal, but then the anæsthesia would gradually become very profound and lead finally to death. In fact, of all the experiments in which the dogs received subcutaneously into one place an effective dose of magnesium salts, no animal survived the prolonged, deep anæsthesia into which it gradually passed. The animal would remain for many hours on the same spot and in the same position, and the death was never marked by any struggle. One dog, which received subcutaneously into one place 2 gm. of magnesium sulphate per kilo, and which possibly had a chance of recovery, was utilized for another experimental purpose.[1]

We had more satisfactory results when the injection was distributed in two places,[2] or when the injection was given intramuscularly. For the dog it was quite evident that the absorption from the muscles was better than from the subcutaneous issue.

The following experiments are good illustrations:

Experiment 10. — Male dog, 9300 gm.

2.30 P.M. Injected 65.1 c.c. of a 25 per cent solution of magnesium sulphate, one-half into the muscles of left back and the other half subcutaneously into right side; massaged.

2.37. Vomited.

2.50. Dog flaccid, cannot move, lies on side; stepping on tail — no response. Lid reflex still active.

Stretched on board, head not fixed, femoral artery exposed and blood pressure tracing obtained (see Fig. 5). No sign of pain and no movements whatsoever. Wound sewed up and animal put on floor.

4.15. Dog lies in same position; anæsthesia deeper than ever. No lid reflex.

Next day, dog survived; passed neither urine nor fæces. Five days later had lost nearly one kilo in weight; otherwise apparently normal. Used for another experiment.

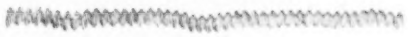
[1] That dog, while in profound anæsthesia, received a fatal dose of strychnine for the purpose of studying the condition of his muscles. The animal responded promptly with strong convulsions.

[2] MELTZER: Journal of experimental medicine, 1900–1901, v, p. 643.

Experiment 11. — Male dog, 8750 gm.

2.55. Injected 61 c.c. of a 25 per cent solution of magnesium sulphate subcutaneously into two separate places (shoulders) ; massaged well.

3.04. On attempting to move tumbles down in a heap.



3.30. Dog placed on board, femoral artery exposed, cannula inserted ; no sign of pain during operation. Blood pressure taken. Frequent slight motions of the animal, each motion causing a short rise of blood pressure (see Fig. 6). Abdomen opened, no sign of pain. Scraping of the parietal peritoneum is resented by movements of the animal ; but no definite struggle. Animal killed.

FIGURE 5. One-half the original size. — Blood pressure tracing from dog (Experiment 10) ninety minutes after receiving an injection of 1.75 gm. magnesium sulphate per kilo. One-half of the quantity was given intramuscularly and the other half subcutaneously. Animal perfectly motionless. Curve and lines as in Fig. 4.

Each animal in the two experiments received 1.75 gm. per kilo. In Experiment 10 one-half was given intramuscularly, whereas in Experiment 11 the entire amount was given subcutaneously but in separate places. Both were in satisfactory states of anæsthesia. However, the differences between the degrees of anæsthesia in the two animals are striking. The anæsthesia in the animal of Experiment 10 (partly intramuscular) was already absolutely satisfactory during the operation and the taking of the blood pressure tracings, but later it became still more profound; whereas the anæsthesia of the animal of Experiment 11 was not profound enough during the taking of the tracing and it soon began to show signs of waking up. The difference finds its clear expression in the blood pressure tracings obtained from the two animals. The tracing from the animal of Experiment 11 is frequently interrupted by considerable elevations brought on by the motions of the animal; while that from the animal

FIGURE 6. One-half the original size. — Blood pressure tracing from dog (Experiment 11) fifty minutes after receiving 1.75 gm. magnesium sulphate per kilo subcutaneously in two different places. Curve and lines as in Fig. 4, time line above base line. Elevations on blood pressure curve correspond to movements of the animal.

of Experiment 10 runs as evenly as if the animal were also under the influence of curare.

The blood pressure of all dogs which were under anæsthesia from the magnesium salts was comparatively low — under 100 mm. mercury. This apparently did not interfere with the final recovery of the animals. Neither did it seem to be connected with the depth of the anæsthesia, since the blood pressure of dogs with light anæsthesia was also comparatively low.

Nearly all dogs which received magnesium sulphate vomited at least once and in most cases a few times; the first vomiting occurred soon after the injection. This happened even when the general effect was slight.

Dogs which received magnesium chloride did not vomit.

In nearly all cases the injection of magnesium sulphate produced no pain. The injection of magnesium chloride seemed to be slightly painful.

Nearly all the injections in dogs produced abscesses, but the solutions used were not sterilized.

In dogs also, as we have seen above in cats, a satisfactory anæsthesia was fully developed before complete muscular relaxation was attained. Furthermore, a complete abolition of pain was doubtlessly present before the conjunctival reflex was entirely gone.

Injection of Magnesium Sulphate in Guinea-Pigs.

A dose of 1.8 gm. per kilo produced in these animals deep anæsthesia after fifteen minutes; but with this dose death followed about fifteen minutes later. A dose of 0.8 gm. per kilo only quieted the animal. Doses between 1.0 and 1.25 gm. per kilo caused more or less complete anæsthesia followed, as a rule, by complete recovery. However, some individual disposition of the animal seems to have something to do with the degree of the effect. The following protocols will serve as illustrations:

Experiment 12a. — Red guinea-pig, 480 gm.

3.27. Injected 2 c.c. of a 25 per cent solution of magnesium sulphate (about 1.0 gm. per kilo) subcutaneously into right flank.

4.30. Remains on side when placed there; conjunctival reflex slight.

5.00. Able again to get legs under him; can still be held up by a leg with only slight movements of legs and head.

5.30. Resists vigorously being placed on back or side; conjunctival reflex active. Complete recovery.

Experiment 12b. — Same guinea-pig eight days later, weighs now 535 gm.

2.55. Injected subcutaneously, left flank, 2.4 c.c. of a 25 per cent solution of magnesium sulphate (1.1 gm. per kilo); massaged.

3.20. Can be placed on side to stay; struggles very slightly when held up by one leg; conjunctival reflex slight.

3.44. Continued to lie on back, completely flaccid; corneal reflex practically absent.

3.57. Turned over on belly and moved about. Complete recovery.

Was used two days later for a similar experiment with an identical result.

Experiment 13a. — Black guinea-pig, 595 gm.

2.37. Injected subcutaneously, right flank, 3 c.c. of a 25 per cent solution of magnesium sulphate (1.25 per kilo); slight massage.

4.30. Turns on belly when placed on side; conjunctival reflex active. Five minutes later remains lying on side.

5.00. Still stays on back as placed; slight movements of head and legs when held up by legs.

5.30. Resists being placed on back. Complete recovery.

Experiment 13b. — Same animal (black) eight days later; weighs now 649 gm.

2.58. Injected subcutaneously, right flank, 3 c.c. of a 25 per cent solution of magnesium sulphate (1.2 per kilo); massaged.

3.20. Resists vigorously being placed on side.

3.44. Can be placed on back, but turns on belly again; conjunctival reflex active.

4.45. Resists attempts to be placed on side.

Animal was practically never in a distinct anæsthetic state.

The red guinea-pig of Experiment 12 was completely anæsthetized three times with doses not above 1.1 gm. per kilo; whereas the black animal of Experiment 13 already showed a greater resistance in the first experiment with a dose of 1.25 gm. and was practically not anæsthetized by a dose of 1.2 gm. per kilo.

We have to bear in mind, however, that in guinea-pigs we cannot always be sure that a subcutaneous injection was not partly intramuscular, and hence the variations in the effects with the same doses.

The anæsthetic effect as a rule set in late, often as late as an hour after the injection, and did not last very long. When the effect took place early, the anæsthesia usually had a fatal termination. No intramuscular injections and no magnesium chloride was tried on guinea-pigs.

In no case did the subcutaneous injection of magnesium sulphate produce any diarrhœa.

No abscesses developed in guinea-pigs.

Injections in White Rats.

Only magnesium sulphate was tested and only by subcutaneous injections.

A dose of 1.25 gm. per kilo had very little effect. 1.50 gm. per kilo had a somewhat better effect and never produced death, but the anæsthesia was far from being complete. Doses of 1.75 gm. per kilo produced complete anæsthesia ten to fifteen minutes after injection; but in five such experiments only two rats survived the deep narcosis; in three animals, the anæsthesia terminated in death half an hour after injection.

Experiment 14. — White rat (red mark on head), 196 gm.

3.20. Injected subcutaneously, left side, 1.2 c.c. of a 25 per cent solution of magnesium sulphate (175 gm. per kilo); massaged.

3.38. Can be placed on back or side to stay; conjunctival reflex absent; no response to pinching tail.

3.50. Respirations labored, slow.

4.15. Turned on his belly, crawling around. Recovered.

Experiment 15. — White rat (blue mark on head), 235 gm.

3.24. Injected subcutaneously, left side, 1.6 c.c. of a 25 per cent solution of magnesium sulphate (1.75 gm. per kilo); massaged.

3.38. Can be placed on back to stay; no response on pinching tail; conjunctival reflex absent.

3.50. Dead; a few moments before heart beats could be distinctly felt, while the respiration had already stopped.

No abscesses developed in the surviving animals.

No diarrhœa followed any injection.

Injections of Magnesium Salts in Frogs.

A dose of 1 c.c. of a 25 per cent solution of magnesium sulphate or of 19 per cent magnesium chloride injected into one of the lymph sacs of a medium-sized frog will cause complete paralysis of the animal within half an hour after the injection, from which it does not recover. The respirations become gradually slower and shallower until they stop entirely. About that time all voluntary and reflex movements completely disappear. The heart beats, which also become slower soon after the injection, stop entirely about two or three hours later. The above dose, with which we started our experiments, is a very large one; we hardly were aware that it amounted to about 8 gm. per kilo frog! Doses of 1.5 to 2.0 gm. per kilo frog can produce

a complete immobility from which the animal can fully recover sooner or later. The following experiments will serve as illustrations:

Experiment 16. — Frog, 37 gm.

2.30. Injected into ventral lymph sac 0.25 c.c. of a 25 per cent solution of magnesium sulphate (1.5 gm. per kilo).

3.00. Frog lying flat on back as placed; does not move away when pushed; hangs flaccid when lifted by leg. No corneal reflex.

5.00. Turns over actively when placed on back; jumps when pushed.

Next day seems perfectly normal.

Experiment 17. — Frog, 24 gm.; 80 respirations to the minute.

4.00. Injected into dorsal lymph sac 0.19 c.c. of a 25 per cent solution of magnesium sulphate (2.0 gm. per kilo).

4.20. Unable to turn over when on back.

4.35. Flaccid, when raised by leg; no struggle.

5.00. Legs extended, flaccid; 60 respirations to the minute, very shallow.

Next day recovered and normal.

Subcutaneous Injections of Magnesium Sulphate in Fowls.

We made only a few experiments on fowls for the purpose of bringing out the chief point of our contention. Since, however, no experiments were ever made, at least as far as we know, with injections of magnesium salts into fowls, we may be permitted to reproduce here all the protocols of these few experiments.

Experiment 18. — Rooster, 2230 gm.

2.54. Injected subcutaneously, left side, 13.4 c.c. of a 25 per cent solution of magnesium sulphate (1.5 gm. per kilo). Animal responds to each touch with a remonstrating cackle.

3.10. Sits on floor; beak touching floor; quiet; a push causes no cackling.

3.19. Can be placed on back to stay; respiration slow and labored; touch on cornea does not produce the familiar rapid contraction of the third lid.

3.50. In a deep state of anæsthesia; ether applied to nares does not inhibit respiration.

4.20. Clear thin fluid coming from the beak; a large amount of clear fluid issues from the cloaca.

4.48. Death. No preceding struggle.

Experiment 19a. — Pigeon, 280 gm.

2.50. Injected subcutaneously, right side, 1.7 c.c. of a 25 per cent solution of magnesium sulphate (1.5 gm. per kilo).

(Skin very thin, tore, some of the salt escaped.)

3.06. Pigeon still stands erect; no change.

3.10. Seems a trifle unsteady on legs.

3.19. Crouches now.

3.30. Cannot be placed on back; struggles to regain position.

3.38. Can be placed on back to stay; touch of cornea causes twitch of lid.

3.50. Deep anæsthesia. Ether does not stop respiration.

3.54. Turned over from position on back.

4.48. Attempts to fly.

Next day perfectly well.

Experiment 19b. — Same pigeon two days later; lost weight, 258 gm.; otherwise apparently well.

5.02. Injected subcutaneously, left side upper chest, 1.0 c.c. of a 25 per cent solution of magnesium sulphate (1.00 gm. per kilo); no mishap. Until 6.10 pigeon standing erect, no sign of weakness. Injection had no effect.

Experiment 19c. — Same pigeon four days later; about same weight — 260 gm.

3.05. Injected partly intramuscularly, upper right chest, 1.6 c.c. of a 25 per cent solution of magnesium sulphate (1.5 gm. per kilo).

3.30. Lying on side; no lid reflex; respiration slow and labored; placed on back to stay.

4.00. Still on back, can be raised by one leg, but wings do not hang down.

4.10. Flopped over on legs, but, unable to stand, rests on table, bill touching it.

4.30. Lying on side, bill touching table. Tossed into air, made ineffectual attempts to fly. Seems to wake up more; can no longer be placed on back, slight lid reflex.

5.35. Sitting, but legs do not seem to be able to bear weight of body.

Next day normal.

Experiment 20a. — Chicken, 1590 gm.

4.58. Injected subcutaneously, left chest, 6.4 c.c. of a 25 per cent solution of magnesium sulphate (1.0 gm. per kilo).

5.35. Can no longer stand; sits; can be placed on side.

5.50. No change; large amount of fluid passed from cloaca.

6.10. Again a good deal of fluid passed from cloaca. Otherwise no change.

Next day normal. Had henceforth loose stools.

Experiment 20b. — Same chicken; lost weight, now 1548 gm.

3.02. Injected subcutaneously, right chest, 9.3 c.c. of a 25 per cent solution of magnesium sulphate (1.5 gm. per kilo).

3.20. Drowsy; prefers to squat with eyes closed.

3.33. Squatting on table; passed a good deal of clear fluid from cloaca.

4.00. Again passed a good amount of fluid. Can now be placed on side to stay; beak on floor.

4.30. No change; lid reflex active.

5.25. Can still be placed on back, but turns over after short time.

Next day apparently well.

These experiments on fowls have demonstrated that about 1.0 gm. of magnesium sulphate per kilo had either no effect, as in the pigeon, or very little effect, as in the chicken; and further that 1.5 gm. per kilo might produce a moderate anæsthetic effect, as in the chicken, or a profound anæsthesia with perfect recovery, as in the pigeon (partly intramuscular), or a profound anæsthesia with a fatal termination, as in the rooster.

In other words, magnesium sulphate can produce a profound anæsthesia, leading either to perfect recovery or to death, without any symptoms of excitation.

Summary.

We shall now briefly recapitulate our results. In the different species of animals with which we have experimented, rabbits, cats, dogs, guinea-pigs, white rats, frogs, and fowls, we found without any exception:

1. *That a certain dose of magnesium sulphate will produce a deep, often long-lasting anæsthesia with complete relaxation of all the voluntary muscles and abolition of some of the less important reflex activities, which anæsthesia terminates in perfect recovery.*

2. *That a large dose of magnesium salt will produce a profound anæsthesia and general paralysis which leads sooner or later to a calm death without being preceded or accompanied by any symptoms of excitation.* Furthermore, in all of the many experiments we have made with magnesium sulphate, *not a single instance was observed in which that salt produced an increase of excitation;* on the contrary, *any effect which this salt produced was invariably in the direction of a reduction of excitation or of its complete temporary or permanent abolition.*

The same was true also of magnesium chloride in the instances in which it was tested.

With the exception of cats, for all animals a dose of 1.0 gm. of magnesium sulphate per kilo or less had very little effect; a dose of a little over 2 gm. per kilo brought on a fatal anæsthesia. The doses lying between 1 and 2 gm. per kilo animal were the ones which produced more or less complete anæsthesia with recovery. In one species of animal the anæsthetic dose lay nearer the 1 gm. per kilo, and in another species it was nearer the 2 gm. per kilo limit. In general it can be stated that for nearly all animals the dose of magnesium sulphate which can produce anæsthesia with recovery by subcutaneous injection is 1.5 gm. per kilo animal.

For cats it was found that 0.9 per kilo was about the anæsthetic dose for an adult animal. Doses of magnesium sulphate below 0.9 per kilo produced but little effect, and the anæsthesia following a larger dose had a fatal termination. Younger animals seemed to stand a larger dose.

Intramuscular injections, or the injections of divided doses into several places, have favored in some instances the onset of the anæsthetic effect.

We have to mention here that the impression we gained from all experiments was that the animals which urinated frequently had a better chance for recovery. Early urination seemed also to prevent the full development of anæsthesia. Furthermore it seemed that magnesium sulphate after subcutaneous injection acted as a diuretic. Urination probably carried off some of the salt and prevented its fatal accumulation in the blood.

In cats and especially in dogs the injection of magnesium sulphate produced in many instances vomiting or retching. This occurred long before the onset of anæsthesia, and in some instances even when no anæsthesia followed. In the experiments with magnesium chloride no vomiting was observed.

Except in the one chicken we have never seen that the subcutaneous injection of magnesium sulphate ever led to an immediate or late appearance of diarrhœa, or even simply to more frequent stools. In two or three instances in which the abdomen was opened during the experiment or immediately upon its termination there was some fluid and gas present in the gut, but especially in the small intestines. This observation deserves attention with regard to the older and recently renewed claims that subcutaneous injections of magnesium sulphate act as a purgative.

Subcutaneous injection of a 25 per cent solution of magnesium

sulphate seemed to produce hardly any pain in the animals.[1] Magnesium chloride solutions (19 per cent) seemed to be more irritating.

The subcutaneous injection of large quantities of the salt solutions produced abscesses in dogs which healed promptly. In these experiments, as already stated above, no aseptic precautions were taken. In the many injections given to rabbits (with one or two exceptions after intramuscular injection), guinea-pigs, white rats, fowls, and even cats, abscesses were very rarely observed. It remains to be seen in future studies whether by sterilization and asepsis such abscesses can be avoided.

We may in this connection perhaps be permitted to add that the exclusive object of this study was to establish the essential principle, namely, that magnesium salts possess strong anæsthetic powers. The practical application of this new principle was not taken into consideration during the present study.

The fatal effect of the magnesium salts was produced in the first place by paralysis of respiration. It became slower and somewhat labored and with active expirations even in the non-fatal states of anæsthesia. In the fatal anæsthesias the respiration gradually became very slow and very shallow, until finally it stopped entirely. The heart usually continued beating for a while after the cessation of respiration. The institution of artificial respiration at this stage, however, never resuscitated the animal.

In rabbits and cats the blood pressure during the anæsthesia was very little depressed. In dogs it appeared to be considerably lowered. However, it did not prevent prompt recovery.

It deserves to be mentioned especially that in rabbits, even during deepest anæsthesia by magnesium salts, the application of ether to the nose inhibited the inspiration immediately. In anæsthesia by ether or chloroform this occurs only at the beginning, but not when the animal is already fairly under the influence of the anæsthetic. We shall for the present not enter into a full discussion of the interpretation we are inclined to give to this phenomenon. We shall only remind that the stoppage of respiration by ether, etc., is generally considered as an act of reflex inhibition of inspiration by means of stimulation of the peripheral ends of the second branch of the trigeminus. We shall then state our observation in the following

[1] One of us received twice, subcutaneous injections of small doses (1.0 and 1.5 c.c.) of the 25 per cent solution of magnesium sulphate. It produced practically no pain or irritation worth mentioning.

words: Magnesium salt, which reduces or temporarily abolishes (inhibits) many reflex acts, does not interfere with the trigeminal reflex-inhibition of respiration.

Finally, in cats and also in dogs a state of deep anæsthesia, absence of pain sensation, and complete muscular relaxation can be fully developed before the stage of complete abolition of the conjunctival reflex is reached. The latter stage, especially in cats, seems to border on the danger line. Furthermore, in these animals the stage of complete anæsthesia (absence of the sensation of pain) seems to be reached before there is a complete abolition of all voluntary movements. These animals could be operated upon without any sign of pain or resistance while they were still able to move about somewhat.

A STUDY OF THE METABOLISM IN OSTEOMALACIA.

By J. E. GOLDTHWAIT, C. F. PAINTER, AND R. B. OSGOOD AND F. H. McCRUDDEN.

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FOR several years the authors have been making a study of the metabolism in certain bone diseases. Opportunity was recently offered to study osteomalacia in a woman sixteen years old, who had been crippled for several years as the result of this disease.

Two experiments were carried out; the first lasted eight days, the second fourteen days. After the first experiment the ovaries of the patient were removed as a therapeutic measure. The second experiment was performed a few months later, and serves to show the effect of castration on metabolism.

METHOD.[2]

In each experiment the urine was analyzed every day. The food and fæces were analyzed for the whole period. In the first experiment the metabolism of calcium, magnesium, phosphorus, sulphur, and nitrogen was studied; in the second experiment only that of nitrogen, sulphur, and calcium oxide.

The patient fasted from noon of the day before the experiment began until the following morning. Half an hour before breakfast of this morning the patient was given a mixture consisting of 10 gm. charcoal, 10 gm. acacia, and 60 c.c. peppermint water in order to separate the stools belonging to the experimental period. The experiment was ended by the patient fasting from noon on the last

[1] A part of the expenses of this research were contributed by the Proctor Fund.

[2] The details of the methods used will be found more fully described in previous papers. See GOLDTHWAIT, PAINTER, and OSGOOD: American medicine, 1904, vii, pp. 547 and 590. Also F. MCCRUDDEN: Journal of medical research, 1903, ix, p. 135; Berichte der 5ten internationalen Kongressen für angewandte Chemie, Berlin, 1903, iv, p. 256.

day of the experiment until the following morning, and the charcoal mixture was given on this morning as before. The fæces were saved from the time when they first appeared black at the beginning of the experiment until they first appeared black at the end of the experiment.

Urine. — The urine was collected from 8 A.M. to 8 A.M. The twenty-four hours' quantity was made up to 1000 c.c. and an aliquot part taken for analysis. The analyses were made in duplicate.

Nitrogen was determined by the Kjeldahl method (5 c.c. urine, 25 c.c. concentrated sulphuric acid, 5 c.c. copper sulphate, 10 gm. potassium sulphate).

The phosphorus was determined by a gravimetric modification of the method of Neumann.[1] Instead of determining the phosphorus in the phosphomolybdate precipitate volumetrically, it was determined in the usual gravimetric way by redissolving the phosphomolybdate with ammonia, and precipitating with magnesium mixture.

The calcium was determined by the method given on page 746 of Neubauer and Vogel's book on urine analysis.[2] This method has been found to give very good results when compared even with such an accurate method as that of Richards, McCaffrey, and Bisbee.[3]

The determination of magnesium was carried out by the method given in Neubauer and Vogel's book on page 748.

The sulphur was determined by a method used in this laboratory. 50 c.c. of urine was evaporated to dryness in a nickel crucible after addition of sodium peroxide. The residue was moistened with water, a little more sodium peroxide added, and the mixture again evaporated to dryness. This was repeated a few times, and then the sodium peroxide was brought to quiet fusion for a few moments. When the mixture was completely oxidized, it was allowed to cool, and then dissolved in water containing a little bromine. After acidification with hydrochloric acid the sulphuric acid was precipitated and weighed as barium sulphate in the usual manner.

Food. — The patient was allowed to eat what she wanted, except that non-homogeneous food was excluded.

Liquid food was mixed as thoroughly as possible, a certain volume

[1] NEUMANN: Zeitschrift für physiologische Chemie, 1902, xxxvii, p. 115.

[2] NEUBAUER and VOGEL: Analyse des Harns, Wiesbaden, 1898.

[3] RICHARDS, MCCAFFREY, and BISBEE: Proceedings of the American Academy of Arts and Sciences, 1901, xxxvi, p. 377.

given to the patient, and the same volume taken for analysis. Solid food was mixed by cutting into small pieces. The patient received a certain weight, and the same weight was taken for analysis. The sugar and salt used were determined by weighing a small quantity at the beginning of the experiment and determining what was left at the end. All the food taken for analysis was mixed together, evaporated to dryness on the water-bath, ground to a fine powder, thoroughly mixed and weighed and sampled for analysis as described in previous papers. Analyses were made in triplicate.

Nitrogen was determined by the Kjeldahl method.

Phosphorus was determined by the gravimetric modification of Neumann's method.

For the determination of calcium and magnesium, an aliquot part of the food was incinerated, the ash dissolved in a dilute solution of hydrochloric acid, and the calcium and magnesium determined by the method used for the urine.

For the determination of sulphur the method found best was as follows: An aliquot part of the food (from one to two grams) was mixed with a little water and several grams of powdered sodium hydroxide. A few grams of sodium peroxide were then added little by little with constant stirring until the mass was pasty. The mixture was then warmed to gentle fusion over an alcohol lamp. After complete oxidation the mass was dissolved in water and the sulphur determined as in the urine.

The fæces were thoroughly mixed, evaporated to dryness on the water-bath, and analyzed in the same way as the food.

Experimental Data.

Diet in first experiment. — *1st day.* Breakfast: bread 95.92, milk 50.0 c.c., butter 9.10, tea 180.0 c.c.

Dinner: potato 146.6, bread 115.0, pudding 144.90, tea 180.0 c.c., milk 30.0 c.c., butter, sugar.

Supper: apple sauce 49 45, cheese 39.50, tea 170.0 c.c., milk 30.0 c.c., bread, butter, sugar.

2d day. Breakfast: oatmeal 142.35, milk 100.0 c c., coffee 180.0 c.c., bread, butter, sugar.

Dinner: roast beef 49.75, potatoes 161.0, pudding 88.58, milk 30.0 c.c., bread, butter.

Supper: roast beef 15.32, ice cream 52.63, peaches 63.50, bread, butter.

3d day. Breakfast: oatmeal 110.75, beefsteak 45.45, bread, butter.

Dinner: roast beef 18.75, potato 62.35, rice 95.01, milk 60.0 c.c., bread, butter, sugar.

Supper: cold lamb 27.05, jelly 64.40, ice cream 67.20, bread, butter.

TABLE I.

THE ANALYSIS OF THE URINE, FÆCES, AND FOOD.

Day.	CaO.	MgO.	P_2O_5.	S.	N.
1	0.659	0.1124	1.124	0.1838	6.944
2	0.504	0.0819	0.935	0.1958	5.682
3	0.597	0.1036	0.991	0.2150	5.362
4	0.439	0.0913	1.027	0.2166	6.456
5	0.380	0.0754	1.290	0.1796	6.458
6	0.343	0.0565	0.899	0.1956	5.680
7	0.647	0.0750	1.312	0.1882	8.622
8	0.290	0.0710	1.231	0.1424	5.838
Total (in urine) .	3.859	0.667	8.81	1.517	51.04
Fæces	1.80	1.348	3.56	1.166	11.98
Total excreted .	5.66	2.015	12.37	2.68	63.02
Food	4.56	2.207	12.05	7.15	69.12
Retained by body (gm.) . . .	−1.10	+0.192	−0.32	+4.47	+6.10
Retained, per cent Food = 100%	−24.	+9.	−2.7	+63.	+9.
Weight at beginning, 59.0 lbs.; weight at end, 59.5 lbs.					

4th day. Breakfast: oatmeal 138.80, beefsteak 51.35, tea 160.0 c.c., bread, butter, milk, sugar.

Dinner: roast beef 43.58, potato 156.50, custard 76.90, tea 140.0 c.c., milk 30.0 c.c., bread, butter.

Supper: cold lamb 22.64, tea 168.0 c.c., milk, 30.0 c.c., bread, butter, sugar.

5th day. Breakfast: oatmeal 114.60, beefsteak 30.17, tea 156.0 c.c., milk 60.0 c.c., bread, butter, sugar.

Dinner: roast beef 29.12, potatoes 134.55, berries 102.90, milk 150.0 c.c., sugar.

Supper: roast lamb, 82.79, peaches 29.42, tea 150.0 c.c., milk 30.0 c.c., bread, butter.

6th day. Breakfast: beefsteak 68.93, oatmeal 129.13, tea 150.0 c.c., milk 50.0 c.c., bread, butter.

Dinner: corned beef 62.29, potato 180.07, bread, butter.

Supper: cold beef 51.0, peaches 74.87, tea 143.0 c.c., milk 60.0 c.c., bread, butter.

7th day. Breakfast: beefsteak 59.95, oatmeal 162.45, tea 140.0 c.c., milk 80.0 c.c., bread, butter.

Dinner: roast beef 82.93, potato 176.13, oatmeal 93.10, bread, butter, tea, milk.

Supper: cold lamb 24.34, potato 88.88, bread, butter.

8th day. Breakfast: oatmeal 67.78, tea 150.0 c.c., milk 55.0 c.c., bread, butter.

For whole period: bread 991.3, butter 227.7 gm., sugar 244.1, salt 19.89.

There were several peculiar conditions in this case. There was a very great retention of sulphur, and a considerable loss of calcium; there was a slight retention of nitrogen and magnesium. The calcium seemed to be excreted chiefly in the urine rather than in the fæces, and the magnesium in the fæces rather than in the urine, which is the reverse of the normal condition. The daily excretion of calcium in the urine was very high. The normal is considered to be from 0.12 to 0.18 gm. In this case we had from 0.30 to 0.65 gm. The daily excretion of magnesium was low. The normal is from 0.18 to 0.25 gm. In this case it was only from 0.05 to 0.11 gm.

Diet in second experiment. — *1st day.* Breakfast: oatmeal 75.70, beefsteak 84.35, potato 47.00, bread, butter.

Dinner: roast lamb 27.20, potato 83.1, milk 190.0 c.c., ice cream 50.35, bread, butter.

Supper: lamb 17.90, apple sauce 81.15, cocoa 150.0 c.c., cake 45.3.

2d day. Breakfast: beefsteak 49.2, oatmeal 75.1, bread, butter, milk 40.0 c.c., cocoa 140.0 c.c.

Dinner: beefsteak 34.4, potato 77.5, bread, butter.

Supper: lamb 16.1, bread, butter, cocoa 152.0 c.c., apple sauce 68.5.

3d day. Breakfast: scrambled eggs 64.6, oatmeal 98.4 milk 45.0 c.c., cocoa 140.0 c.c., bread, butter.

Dinner: beefsteak 61.3, potato 97.0, snow pudding 119.2, bread, butter, milk 140.0 c.c.

Supper: beefsteak 51.3, pear sauce 77.0, cake 33.5, bread, butter.

4th day. Breakfast: beefsteak 51.20, oatmeal 119.20, cocoa 140.0 c.c., cream 35.0 c.c., bread, butter.

Dinner: roast beef 44.70, potato 83.75, custard 87.0, bread, butter.

Supper: roast lamb 18.55, jelly 64.0, cocoa 158.0 c.c., bread, butter.

5th day. Breakfast: beefsteak 65.6, oatmeal 125.7, cream 30.0 c.c., cocoa 140.0 c.c., bread, butter.

Dinner: roast beef 49.35, potato 73.55, ice cream 100.90, milk 150.0 c.c., bread, butter, cake 41.0.

Supper: roast lamb 30.18, cocoa 148.0 c.c., peach sauce 96.35, cake, bread, butter.

6th day. Breakfast: beefsteak 58.05, oatmeal 101.9, cocoa 150.0 c.c., cream 30.0 c.c., bread, butter.

Dinner: roast beef 28.70, potato 89.20, bread, butter, gingerbread 72.5, snow pudding 72.85, milk 136.0 c.c.

Supper: roast lamb 31.60, potato 37.35, cookies 15.0, apple sauce 105.35, cocoa 132.0 c.c., bread, butter.

7th day. Breakfast: beefsteak 52.65, oatmeal 108.20, cocoa 150.0 c.c., cream 30.0 c.c., bread, butter.

Dinner: roast lamb 42.6, potato 82.45, ice cream 86.35, bread, butter.

Supper: roast beef 23.4, apple sauce 126.5, cocoa 132.0 c.c., bread, butter.

8th day. Breakfast: oatmeal 116.85, omelette 46.90, cocoa 150.0 c.c., bread, butter, cream 30.0 c.c.

Dinner: beefsteak 55.40, potato 71.45, bread, butter, cake 58.0, custard 140.20.

Supper: omelette 60.75, apple sauce 147.25, cocoa 158.0 c.c., bread, butter.

9th day. Breakfast: beefsteak 61.90, oatmeal 129.75, cocoa 156.0 c.c., cream 30.0 c.c., bread, butter.

Dinner: roast beef 67.90, potato 72.10, pudding 107.10, bread, butter.

Supper: roast lamb 20.40, apple sauce 149.90, bread, butter.

10th day. Breakfast: omelette 63.85, oatmeal 124.25, cocoa 158.0 c.c., cream 30.0 c.c., bread, butter.

Dinner: beefsteak 82.45, potato 130.0, snow pudding 120.7, bread, butter.

Supper: beefsteak 44.80, pear sauce 129.80, bread, butter, cocoa, 126.0 c.c.

11th day. Breakfast: omelette 53.10, oatmeal 136.15, cocoa 154.0 c.c., cream 29.0 c.c., bread, butter.

Dinner: beefsteak 43.55, potato 75.45, custard 142.55, bread, butter.

Supper: beefsteak 51.30, apple sauce 109.85, cocoa 140.0 c.c., bread, butter.

12th day. Breakfast: beefsteak 18.40, oatmeal 131.90, cocoa 152.0 c.c., cream 30.0 c.c., bread, butter.

Dinner: roast beef 31.70, potato 106.95, ice cream 127.35, bread, butter.

Supper: roast lamb 13.50, peach sauce 92.65, cocoa 252.0 c.c., bread, butter.

13th day. Breakfast: cocoa 152.0 c.c.

Dinner: roast beef 16.80, potato 59.25, custard 109.70.

Supper: lamb 11.60, cake 75.0, cocoa 136.0 c.c., jelly 67.35, bread, butter.

14th day. Breakfast: beefsteak 33.55, oatmeal 128.55, cocoa 156.0 c.c., cream 30.0 c.c., bread, butter.

Dinner: milk 200.0 c.c., bread, butter.

Bread, butter, sugar, and salt were weighed for the period.

Bread for the experiment	1600.0 gm.
Butter for the experiment	242.0
Sugar for the experiment	170.05
Salt for the experiment	15.30

From Table II it will be seen that there was a greater retention of nitrogen in this period than in the first. The daily excretion of sulphur was decidedly greater at this time than in the first period, and the retention of sulphur was slightly less. The daily excretion of calcium in the urine was still considerably above normal, but was much lower than in the first experiment. In the first experiment the average daily excretion of calcium in the urine was 0.482 gm. In this experiment it was 0.386 gm. The daily excretion of calcium in the fæces was much less in this experiment than in the first. The total quantity in both cases was 1.80 gm. for the whole period, but the first experiment lasted only eight days, and this experiment lasted fourteen days. Perhaps the most striking difference in the metabolism in the two experiments is the fact that, while in the first experiment there was a loss of 24 per cent calcium by the organism, in this experiment, performed after castration, there was a retention of 28 per cent.

Discussion.

It has been generally held that in osteomalacia there is a decalcification of the bony tissue, a "halisteresis." The only basis for this

view is the low content of inorganic material in the bones in this condition. Normal bones contain from 34 to 44 per cent organic

TABLE II.

THE ANALYSIS OF THE URINE, FÆCES, AND FOOD.

Day.	N.	S.	CaO.
1	5.571	0.2614	0.440
2	5.359	0.2646	0.421
3	5.157	0.2606	0.442
4	6.099	0.2746	0.396
5	4.069	0.2696	0.319
6	7.184	0.2742	0.495
7	7.829	0.2586	0.553
8	5.347	0.2598	0.377
9	6.741	0.2562	0.450
10	8.064	0.2038	0.416
11	6.976	0.2312	0.336
12	7.379	0.2070	0.313
13	6.740	0.2196	0.151
14	5.988	0.1835	0.288
Total (in urine)	88.50	3.425	5.397
Fæces	15.78	1.414	1.80
Total excreta . .	104.28	4.84	7.20
Food	127.0	10.54	10.03
Retained by body (gm.)	22.7	5.70	2.83
Retained, per cent Food = 100%	+18.	+54.	+28.
Weight at beginning, 62.5 lbs.; weight at end, 62.7 lbs.			

material and from 56 to 66 per cent inorganic material.[1] In osteomalacia, on the other hand, the bones contain from 60 to 80 per cent organic material and from 20 to 40 per cent inorganic material.[2]

Other data have recently been obtained, however, which confirm the supposition that in osteomalacia there is a decalcification of the bony tissue taking place. Thus Limbeck[3] and Neumann[4] each, in an experiment of several days' duration, found that there is a loss of calcium by the body in this disease, and our experiment confirms the discovery. The results are shown in the table.

	Limbeck.	Neumann.	Authors.
CaO in urine	1.773	....	3.859
CaO in fæces	3.834	....	1.80
Total excreted	5.607	11.65	5.66
Total in food	2.965	11.26	4.56
Loss	2.042	0.39	1.10

The decalcification is generally attributed to the action of an acid, and especially to lactic acid. This idea is based on the hypothesis that lactic acid will destroy bone, on the discovery of lactic acid in the urine in osteomalacia, and on observations showing a decreased alkalinity of the blood in this condition.

Concerning the action of lactic acid on bones, an experiment of Mörs and Muck[5] indicates that if a bone be put into this acid it will be decalcified to some extent. Mosetig-Moorhof[6] denies this. But in any case it seems doubtful if the experiment can be taken to indicate that the small quantities that might be formed would destroy living bone.

[1] Zalesky: Medizinische-chemischen Untersuchungen v. Hoppe-Seyler, 1886, i, pp. 34 and 39; Gorup-Besanez: Lehrbuch der Chemie, 1874, iii.

[2] Senator: In v. Ziemssen's Handbuch der speciellen Pathologie und Therapie, 1876, xiii, p. 236; Roloff: Virchow's Archiv, 1866, xxxvii, 433; Huppert: Archiv der Heilkunde, 1867, viii, 345.

[3] Von Limbeck: Wiener medizinischen Wochenschrift, 1894, xliv, pp. 737, 794, and 844.

[4] Neumann: Archiv für Gynäkologie, 1896, li, p. 130.

[5] Mörs und Muck: Deutsches Archiv für klinische Medizin, 1869, v, p. 485.

[6] Mosetig-Moorhof: Centralblatt für Chirurgie, 1885, p. 12.

Although lactic acid has been found in the urine in osteomalacia by many observers, yet it is not always present in this condition. Hofmann,[1] among others, has failed to find it in two cases. Further, we know that lactic acid is often found in the urine in conditions other than osteomalacia.

A decrease in the alkalinity of the blood in osteomalacia was found by titration methods by Renzi,[2] Jaksch,[3] Issmer,[4] Truzzi,[5] and Eisenhart,[6] and this has been taken as an indication of the presence of an acid. Fehling[7] and Limbeck,[8] who likewise used titration methods, found the alkalinity of the blood normal. Recent determinations of the alkalinity of the blood by accurate electrochemical methods have shown that the values obtained by titration methods are altogether incorrect, and have no relation to the true alkalinity of the blood.[9] Further, the formation of an acid in the tissue would not be shown by analysis of the blood, for such an acid would be immediately neutralized just as the carbonic, sulphuric, lactic acid, and other acid bodies constantly forming are continuously being neutralized.

The above data seem to establish the fact that the bones are poor in calcium in osteomalacia, and that calcium is continuously being lost through the urine. The hypothesis that the process of decalcification is a solution of calcium by an acid, similar to the solution which takes place when a dead bone is placed in hydrochloric acid, does not seem to be justified.

While it seems to be generally believed that in osteomalacia there is a loss of calcium by the bones, some writers maintain that this alone takes place, others that the calcium lost is replaced by osteoid tissue. Thus, according to the view of Lobstein,[10] Virchow,[10] and many others, the difference between rickets and osteomalacia lies in the fact that in the latter there is merely a decalcification, while in

[1] Hofmann: Centralblatt für innere Medizin, 1897, xviii, p. 329.

[2] Renzi: Virchow's Archiv, 1885, cii, p. 218.

[3] Von Jaksch: Zeitschrift für klinische Medizin, 1887, xiii, p. 360.

[4] Issmer: Archiv für Gynäkologie, 1889, xxxv, p. 310.

[5] Truzzi: Centralblatt für Gynäkologie, 1892, xvi, p. 574.

[6] Eisenhart: Deutsches Archiv für klinische Medizin, 1892, xlix, p. 156.

[7] Fehling: Archiv für Gynäkologie, 1891, xxxix, p. 171.

[8] Von Limbeck: Grundriss einer klinischen Pathologie des Blutes, Jena, 1892, p. 58. Wiener klinische Wochenschrift, 1894, xliv, pp. 737, 794, and 844.

[9] R. Hober: Archiv für die gesammte Physiologie, 1900, lxxxi, p. 522.

[10] Cited by O. Vierordt in Nothnagel's Specielle Pathologie und Therapie, Part VII, ii, p. 124.

rickets there is at the same time a new formation of osteoid tissue. The histological researches of Cohnheim [1] and Hanau,[2] on the other hand, led these authors to the view that there is no essential difference between rickets and osteomalacia in this respect, there being a new formation of osteoid tissue in both cases. Kossowitz,[3] Pommer,[4] and Vierordt,[3] too, take this view. It must be admitted, however, that the histological researches upon which these conclusions have been based have not been conclusive.

If we use the method of calculation of Luthje and Berger [5] to interpret our results, they can be seen to indicate that Cohnheim and Hanau are correct in believing that the inorganic material being lost is replaced by organic material. Bone contains 52.8 per cent calcium oxide and 38.7 per cent phosphorus pentoxide. Then, if we assume that in our experiments the calcium oxide lost by the organism comes chiefly from the bones, and this seems probable from the comparative poverty of other tissue in calcium oxide, and seems especially true in the condition of osteomalacia, the 1.10 gm. calcium oxide lost corresponds to 0.81 gm. phosphorus pentoxide. The observations of Levy,[6] too, who found that calcium and phosphorus are lost in this disease in the ratio in which they occur in bone, confirm this assumption. But as only 0.32 gm. of the phosphorus pentoxide appears in the excreta, 0.49 phosphorus pentoxide is retained in the organism in some form. Flesh contains 0.47 per cent phosphorus pentoxide and 3.4 per cent nitrogen,[5] so that the 6.10 gm. nitrogen retained corresponds to 0.84 gm. phosphorus pentoxide. We can see, then, that even if we do not take into consideration the fact that the magnesium retained would require some phosphorus pentoxide to combine with it as magnesium phosphate (0.23 gm., if it is used to replace the calcium phosphate in the bone), there is still too little phosphorus pentoxide retained to make ordinary muscle and organ flesh. This would seem to indicate that the nitrogen retained does not go to form flesh but to form some material which is poorer

[1] Cohnheim: Allgemeine Pathologie, i, p. 516.

[2] Hanau: Fortschritte der Medizin, 1892.

[3] Cited by O. Vierordt, in Nothnagel's Specielle Pathologie und Therapie, Part VII, ii, p. 124.

[4] Pommer: Untersuchungen über Osteomalacie und Rhachitis, Leipzig, 1885, p. 301.

[5] Luthje und Berger: Deutsches Archiv für klinische Medizin, 1904, lxxxi, p. 278.

[6] Levy: Zeitschrift für physiologische Chemie, 1894, xix, 239.

in phosphorus pentoxide, and, from the enormous retention of sulphur, richer in sulphur than flesh. The material in the body which fills those requirements best is that making up the organic matrix of bone and cartilage; this contains little or no phosphorus, but is very rich in sulphur. This would suggest that we have not alone a decalcification, but also a replacement of the inorganic material of the bone by organic material.

The very great retention of sulphur is important in this connection. It is greater than can be accounted for by assuming that it is used to form any of the organic constitutents of bone or cartilage, for, although these substances are rich in sulphur, yet there is not enough nitrogen retained to correspond to all the sulphur. In this connection, too, may be mentioned the observations of Schmidt[1] and Langendorff and Mommsen,[2] who found that osteomalacia bones do not yield any gelatine. These observations tend to confirm the belief that the inorganic material lost is replaced by osteoid tissue, but indicate that the new material is somewhat different in composition from normal osteoid tissue. The substance giving gelatine may not be entirely missing, as shown by Levy.[3]

It seems probable that the calcium lost is replaced not alone by organic osteoid tissue but in part also by magnesium. According to Gorup-Besanez[4] the inorganic material of normal bone consists of 1.04 per cent, and according to Gegenbauer,[5] of 1.75 per cent magnesium phosphate. In osteomalacia, on the other hand, Huppert[6] found that 9.6 per cent, and Chabrié[6] 26.9 per cent, of the inorganic material is magnesium phosphate. From our Table I it will be seen that 9 per cent of the magnesium in the food was retained by the body. The same reasoning, then, that leads us to the conclusion that the bones are losing calcium will lead us to the conclusion that they are gaining magnesium in osteomalacia.

We come next to the effect of castration. The effect on the calcium metabolism is most marked. Table (A) shows the results in a case of Neumann's and in our case. Both were moderately

[1] SENATOR: In v. ZIEMSSEN's Handbuch der specielle Pathologie und Therapie, 1879, xiii, p. 236.

[2] LANGENDORFF and MOMMSEN: Virchow's Archiv, 1877, lxix, p. 412.

[3] LEVY: Zeitschrift für physiologische Chemie, 1894, xix, 239.

[4] GORUP-BESANEZ: Lehrbuch der Chemie, 1874, iii.

[5] GEGENBAUER: Anatomie des Menschen, 1883, p. 98.

[6] CHABRIÉ: Les phénomènes chimiques de l'ossification, Paris, 1895, p. 65.

severe cases, in which the organism was losing calcium. After castration there was a retention of calcium and a marked improvement in the condition. Table (B) shows the results of another experiment of Neumann's. This was a very severe case, and the condition was not improved by castration. The indication is that when the disease has lasted a very long time the organism can finally lose no more calcium. When this condition is reached the morbid tendency cannot be corrected by castration.

	A.				B.	
	Neumann.[1]		Authors.		Neumann.	
	Before.	After.	Before.	After.	Before.	After.
CaO in food	11.26	12.98	4.56	10.03	16.48	3.65
CaO excreted	11.65	7.20	5.66	7.20	15.90	3.14
	−0.39	+5.78	−1.10	+2.83	+0.58	+0.51

[1] Neumann: Archiv für Gynäkologie, 1896, li, p. 130.

The effect of castration on the sulphur metabolism is not so striking as the effect on the calcium metabolism, but still there is a tendency toward correction. Before castration, in our experiment, the ratio of the sulphur retained to that of the nitrogen was as 73 : 100; after castration the ratio was 25 : 100, which, though still high, is much nearer normal.

Our work would seem to indicate, then, that in osteomalacia there is at first a decalcification of the bony tissue; that the calcium is in part replaced by magnesium, but probably chiefly by an organic substance rich in sulphur, poor in phosphorus, similar to but not exactly like the normal organic matrix. We hope to further confirm this statement by a direct analysis of bone from a case of osteomalacia.. If castration is performed during this period, the decalcification process is checked, and what has been lost is replaced. From Neumann's work we can see that if the condition has lasted until it is very severe, the decalcification process finally comes to an end, but that after this castration does not restore the normal calcium metabolism.

Our patient is now in a very much improved condition, and we hope to study the metabolism again to see if it has become normal. It is to be regretted that the phosphorus pentoxide and magnesium oxide metabolism was not studied in the second period. We hope to study one more case more fully, and confirm our results, and to make complete analyses of the bones in this condition especially for the sulphur and organic bodies present.

AN IMPROVED CAGE FOR METABOLISM EXPERIMENTS.[1]

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NEARLY three years ago I exhibited, at the first meeting of the Society for Experimental Biology and Medicine, a cage designed for metabolism experiments, especially on dogs. A year later photographs of the same cage were shown at a meeting of the American Physiological Society. The cage referred to was similar to the one employed in my first metabolism experiments, which were carried out in the Sheffield laboratory of physiological chemistry under Professor Chittenden's general supervision,[2] but was the fourth I have had occasion to design since, and was the outcome of continuous experiences in metabolism investigations. The original cage had only two movable parts, — a hinged top and a sliding drip pan, — and in some respects, therefore, was rather inconvenient. Each succeeding cage had a greater number of movable parts. Various other mechanical improvements in each added to the convenience of the operator and, besides, favored more complete and perfect collection of the excreta.

No description of any of the cages was ever published, although the following brief reference to the latest design appeared in Volume I of the Proceedings of the Society for Experimental Biology and Medicine: A cage specially designed for experiments on dogs was shown. The parts are so adjusted as to favor the collection and separation of fæces, urine, and cast-off hair. The improvements consist mainly of mechanical devices suggested by experimental experiences of the past few years in metabolism work, all of which are designed to

[1] GIES: Proceedings of the Society for Experimental Biology and Medicine, 1904, i, p. 14; also, Science, 1903, xvii, p. 469, American medicine, 1903, v, p. 708, and GIES and collaborators: Biochemical researches, 1903, i, p. 62. See, also, Proceedings of the American Physiological Society, December, 1903; This journal, 1904, x, p. xxii.

[2] CHITTENDEN and GIES: This journal, 1898, i, p. 1; also, GIES and collaborators: *Loc. cit.* Reprint No. 16.

insure quantitative accuracy as well as comparative convenience in the collection of excreta.

Numerous requests have since been made for descriptions and photographs of the improved cage. It has been found to serve excellently in this laboratory the various purposes for which it was designed. These facts have led me to present here several views of the cage and the following description of it.

General construction. — The general features in the construction of the cage illustrated on pages 405–409 may be described briefly as follows: The extreme sizes of the frame are: length three feet six inches, width two feet four inches, height five feet six inches. A hinged front door is situated at the top. It is one foot high and swings outward and down. The top of the cage is hinged at the rear and swings upward and back. Stay-joints on one side make it convenient to fix the open positions of the hinged top at angles of about 45° or 90° (see p. 406). Small iron bars are inserted across the top of the cage, in the door, and in the remaining three sides for a distance of one foot from the top. Matched and beaded ceiling forms the panels, which surround a movable, zinc-lined, thin-walled, bottomless box. The latter rests on a sliding frame, to which is fastened a stout wire net.

The thin-walled, bottomless box, or lining, is one foot six inches deep and the bottom edge is sharply bevelled. The zinc covering is continued three or four inches high on the *outside* of the box, and is secured there by nailing; it also extends well over the top edge of the box to cover the narrow space between the box and the frame. A metal ring, connected with a rod and terminal hook, is attached to the top edge of the movable box at the middle of each end. The rod is of sufficient length to reach the top rail of the frame, where the hook engages a support, when the rings are grasped and the box is raised high enough to allow the sliding frame, upon which it rested, to be pulled out. Eight thin, hardwood strips are attached to the outside of the box to keep it securely though not tightly in place on the sliding frame, and to favor its easy movement up and down. The box is durable, though light in weight.

The upper surface of the sliding frame, which is grooved at the sides for the slides that support it, has a raised border and a bevel upon which the zinc-lined box rests. The sliding frame is covered with sheet-zinc, and a galvanized wire net is tightly secured across the *bottom* of the sliding frame. The meshes of the network are about

three-eighths of an inch square. The animal rests on the net. Immediately below the network is a drip pan of galvanized iron, which is supported on slides, and is easily moved in and out.

A long narrow shelf with a raised border is placed about a foot below the lowest point, that is, the outlet, of the drip pan, and is supported at each end by a removable iron rod in such a way that the shelf may be easily pushed forward and backward. The rods penetrate the legs of the frame, and are easily adjusted at any height under the pan. A set of folding steps is arranged at the front.

Cage closed, steps folded up and sliding shelf under the outlet of the drip pan.

The construction of the cage is shown in the accompanying figures and by the diagram of a transverse section of the cage.

Remarks on the utility of the cage. — This form of cage is particularly well adapted for use in careful experiments that may be continued for long periods, and in which catheterization is to be entirely or almost wholly avoided. It cannot be used for respiration work. Whatever exercise the animal needs must be taken outside of the cage.

Urine, fæces, cast-off hair, and scurf can be completely collected and readily separated. The fæcal matter usually remains on the network. Fine wire netting fastened to the open sides and top of the cage will insure complete retention of any cast-off hair (when *special* quantitative accuracy is desired), that may tend to pass through the top of the cage, without interfering materially with the circulation of air through the cage. As a rule, nearly all of the cast-off hair and scurf are found on the drip pan. The hair can be readily taken up with an ordinary test-tube brush, to which it adheres intimately, if the brush be revolved on its axis as it is moved lightly over the surface of the pan. Scurf may also be separated and collected by this process, or by sieving the hairy mixture containing it. In cases of diarrhœa such separations cannot be made satisfactorily by any process.

The dimensions given for the particular cage described here have been found satisfactory for cats and dogs of ordinary sizes. It is obvious that cages of the same design may be made of larger or smaller dimensions, to suit the convenience of the operator or the conditions of his experiments. Smaller cages of this general type would be found very satisfactory for metabolism experiments on rabbits, guinea-pigs, and rats.

Cage open, with movable lining suspended and steps lowered; sliding frame with wire mesh, also drip pan, pulled out somewhat; sliding shelf pulled forward.

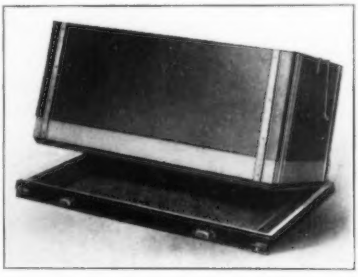
The legs of the frame of this cage were made relatively long, so that the urine in the collecting vessel would be visible from practically all parts of the room, and also in order that the operator might obtain the excreted urine with the greatest possible ease. The same result would be accomplished by placing a short-legged cage on a table. The space under the cage can be used to advantage, however, as the illustrations indicate.

Because of the unusual height of the cage, a small animal cannot ordinarily be seen in it unless the observer is comparatively close to it and able to look over the side. When it is desired to see the animal at any moment from any part of the room, a mirror properly suspended above the cage will gratify all desires in this connection. This arrangement also favors careful observation of the animal at a distance, under conditions that avoid such disturbances as may result from the approach of the observer.

The folding steps serve not only as a seat for the experimenter when the urine is examined in the collecting vessel or when the latter and its contents are removed from the shelf, but are serviceable at all times when the operator wishes to reach any part of the interior of the movable box, or to feed or take hold of the animal. The height of the cage is such that a man of average stature can touch all parts of the cage, feed the animal, remove fæces,

etc., without necessarily using the steps, although for a few operations, particularly the removal of the animal, the steps are usually indispensable.

The drip pan is so arranged that numerous modifications in the method of collecting the urine are possible. The sliding shelf, which is intended to hold the collecting vessel (if the latter is not attached directly to the drip pan or connected with it in some other way) is also a convenient resting-place for various pieces of apparatus that are commonly employed in metabolism experiments. The shelf also favors the use of electrical apparatus to ring out the time of elimination of urine-fractions, in experiments in which fractions of the urine must be examined separately and immediately after their natural excretion.



Movable lining and sliding frame with wire mesh in special position outside of cage to show relations they bear to each other.

As has already been indicated, the character of the drip pan permits of various devices for the collection of the urine. For this purpose we have usually placed directly below the outlet tube of the drip pan a receiver consisting of two parts: a wide-mouthed bottle of appropriate dimensions; a funnel of corresponding size, with the tube removed[1] (see pp. 405 and 406). The funnel serves not only to direct the urine into the bottle, but, by practically closing the mouth of the bottle, prevents appreciable loss of water by evaporation from the collected urine. The funnel fits the mouth of the bottle just as satisfactorily without the tube as with it, and without the tube it may be tilted through a larger angle, thus favoring adaptability to various conditions of adjustment. A tubeless funnel of proper size is not very readily knocked from the bottle by ordinary means, because of its proneness to return to the vertical position from various angles to which it may have been tilted.

The best place for a receiver of the kind described is a position

[1] The removal of the tube from the funnel is necessitated by the fact that when cast-off hair is washed along from the drip pan in a stream of urine, the hair is apt to clog the tube. If the quantity of urine is fairly large, under such conditions, there may be an overflow of urine from the funnel to the shelf before the accumulated volume can filter into the receiver.

directly under the outlet tube of the drip pan, so that the drip pan opening and the opening into the receiver are in a vertical line. If the circumference of the opening through the funnel is as large as that of the outlet tube from the drip pan, there is never any splashing when the urine gushes into the funnel, or, at all events, no urine splashes over the edge of the funnel. If the height of the bottle and funnel, when the latter is in place, is very slightly less than the distance from the shelf to the lower edge of the outlet tube from the drip pan, either the drip pan or the shelf may be moved in and out without bringing the outlet tube of the drip pan in contact with the funnel of the receiver, when the latter is in the accustomed position on the shelf. Under these circumstances it is impossible to upset the receiver, if the drip pan is pulled out before the receiver has been moved away from its usual position.

When for any reason it is desirable to have the outlet tube in constant contact with the funnel of the receiver, the latter may be moved to one side to permit such contact and the shelf may be raised sufficiently to bring the lower edge of the outlet tube below the upper edge of the funnel. If it be desired, under these conditions, to guard against the possibility of upsetting the receiver by moving the drip pan thoughtlessly before the receiver has been taken away, it is only necessary to arrange to have the lower edge of the tube from the drip pan near the upper edge of the funnel. If these relations of the two are maintained, when the drip pan is pulled out the outlet tube strikes the loosely fitting funnel at its upper edge, the blow merely tilts the funnel sufficiently to allow the outlet tube to glide over it, and after the pan has passed, the funnel remains in the tilted position or, as is usually the case, resumes the upright position. In either position of the funnel the receiver remains closed and undisturbed.

If it is desired to make the distance of extension of the outlet tube into the funnel relatively long, a particularly flexible rubber tube attached to the outlet tube will make this relation possible, without materially increasing the danger of upsetting the receiver by accident through careless withdrawal of the drip pan. A receiver of the kind described has the particular advantage of enabling the operator to remove it instantly and substitute another without delay.

The movable bottomless box, or, in other words, the adjustable lining of the cage, was designed to make *drainage from the lateral surfaces perfect* and to permit the use of a sliding frame with wire network that *would not increase the distance between the animal*

and the surface of the drip pan. The wire netting is stretched across the *under* side of the sliding frame. The net is thus brought close to the drip pan, and, besides, sufficient space is allowed for withdrawal of the frame without disturbance of any fæces that may rest on the wire. The greater the distance between the network and the drip pan, the greater the probable loss of urine from splashing to the intervening parts of a cage that cannot be drained by a drip pan. In the cage under consideration the lower edge of the movable lining rests tightly and perfectly on the frame that holds the wire netting, on lines *below* the upper borders of the sliding frame, and well within the outer limits of the drip pan, so that the distance between the network and the drip pan is relatively slight, and loss from splashing is practically impossible. The fall of the drip pan is slight and starts from the *upper* and *outer* edges, thus bringing the outlet, in the middle, as near the level of the wire netting as can possibly be effected.

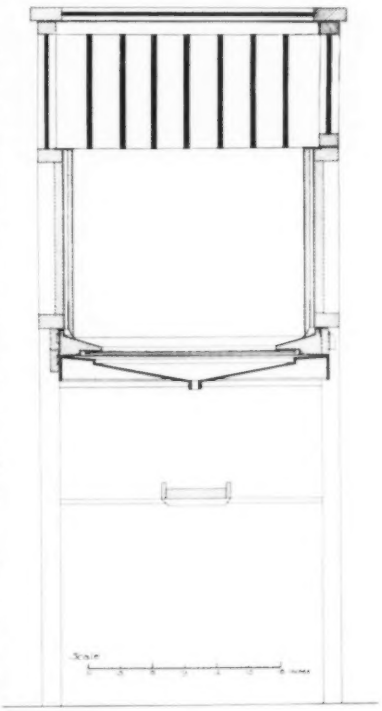


Diagram showing cage in transverse section.

The mobility of the wire bottom favors complete removal of fæcal matter that tends to collect in the angles of the meshes and on the under side of the network. It also favors thorough cleansing of the wire for experiments in which the fæcal matter should not be contaminated in any way. In experiments in which these considerations are of little or no importance, the movable lining likewise offers no advantages. In such cases the frame holding the wire netting could be made stationary, its zinc covering continued up the inner sides of the cage above it, and the movable lining dispensed with.

Slight losses of water from the excreted urine are inevitable in any cage in which elimination of urine takes place naturally. They are

no greater in the described cage than in any other; they are, perhaps, less in it than in many others. The line of contact between the movable lining and the frame holding the wire netting is so perfect that no urine can there be thrown behind the lining. Traces that may occasionally tend to go behind the lining as a result of capillarity at the junction of lining and net holder, can easily be recovered. The bevelled edge of the lining prevents passage by capillarity of urine beyond the very narrow line of contact and also facilitates

Volume thrown into the cage.	Volume delivered into the collecting vessel.	Volume retained on the wire mesh and drip pan.
c.c.	c.c.	c.c.
510	501	9
309	301	8
191	184	7
110	107	3
64	61	3
44	42	2
26	25	1

removal, with a small wet sponge or test-tube brush, of any adherent matter, liquid or solid. In cases of exceptional character the elevated border of the frame that holds the netting prevents loss of urine, and also favors complete recovery of any slight residues from evaporated portions, as on the surface of the drip pan itself. As a rule the animal, male or female, urinates directly on the pan, very near the middle of it. The possibilities considered above occur only in unusual cases especially when male dogs persist in urinating against the side of the cage, or directly toward an angle made by the frame that holds the wire network and the movable lining resting on it. None of these considerations need concern the experimenter when female animals are under observation in this cage.

As every one knows, the volume of urine *delivered* to the collecting vessel of a cage is never equal to the volume of urine actually *eliminated*. Some of the urine adheres, of course, to the drip pan and there evaporates, but the *solid* matter thus deposited is easily recovered by an ordinary washing process. In this cage the differ-

ences between the volumes eliminated and delivered are relatively slight, and, as I indicated before, are not due to urine lost mechanically but to water lost by evaporation from the urine retained on the drip pan. I have frequently poured directly on the network, or on the sides of the movable lining of the cage, known volumes of water in streams similar in volume and force to those commonly ejected by male dogs and have never noted a loss of more than 10 c.c. It is usually little if any more than 5 c.c. The summary on the preceding page gives a few data from my notes on this matter, that were obtained in experiments in which splashing effects were purposely exaggerated somewhat.

That the retention on the wire net and drip pan of solid matter from excreted urine is relatively slight in this cage has been repeatedly ascertained. In some recent experiments on dogs, by Messrs. W. N. Berg and W. H. Welker, under the writer's guidance, to ascertain the metabolic effects of ingested and injected bromids of radium and barium,[1] the interior of the cage was washed only at the ends of periods of a week or more in length, instead of daily. Bacterial development on the drip pan and in the collecting vessel was prevented by an occasional sprinkling of finely powdered thymol. The "period cage washings" contained the solid matter of evaporated urine and of some dried fæces; also small amounts of scurf that had been caught in the evaporated urine and held on the pan. The data for nitrogen content of these washings are significant, especially when considered in connection with the remaining figures in the summary on the following page, for which I am indebted to Mr. Berg.

The consistence of the fæces is an important matter in the satisfactory use of any cage. I have already referred to the fact that the employment of bone ash in the diet favors the elimination of relatively hard white fæcal matter such as dogs ordinarily pass when bones are eaten by them.[2] I have never observed any tendency on the part of dogs to eat fæcal matter of this character, and the fairly hard lumpy condition of these masses, especially on cooling and drying, makes their removal from the cage and preparation for analysis very simple.

[1] Berg and Welker (communicated by Gies): Proceedings of the Society for Experimental Biology and Medicine, 1905, ii, p. 89: also Science, 1905, xxi, p. 988, American Medicine, 1905, ix, p. 1030, and Medical News, 1905, lxxxvii, p. 526.

[2] Gies: Proceedings of the American Physiological Society, December, 1903: This journal, 1904, x, p. xxii.

It is my intention to report in detail, later, the advantages that accrue from the use of bone ash as indicated.

The cage may be cleaned perfectly and with great ease, because it can be taken apart so readily and completely. This is obviously a

Period. No.	No. of days in the period.	Total volume of urine collected.	Total nitrogen of the washings.	Average daily volume of urine collected.	Average daily amount of nitrogen in the washings.[1]	Total nitrogen in 5 c.c. of urine (average).
		c.c.	gram	c.c.	gram	gram
1	9	2106	0.2744	234	0.0305	0.0805
2	19	4129	0.7658	217	0.0403	0.0835
3	7	2120	0.1024	303	0.0146	0.0738
4	12	3551	0.4290	296	0.0358	0.0722
5	5	1178	0.3753	236	0.0751	0.0698
6	12	2953	0.7868	246	0.0656	0.0721
7	5	1262	0.5961	252	0.1192	0.0766
8	7	1353	0.1378	193	0.0197	0.0896
9	8	1817	0.1426	227	0.0178	0.0690

[1] These figures cannot indicate accurately the amount of nitrogenous matter that is *daily* dried upon the drip pan. In such experiments each sample of urine that flows along the surface of the pan dissolves a portion of the solid matter from previous deposits. These figures show conclusively, however, that relatively slight quantities of solid matter are ordinarily retained on the drip pan.

consideration of particular importance from more than one standpoint. The excreta cannot come in contact with any other than metallic parts.

In devising some of the parts of the cage I have profited by the advice of our laboratory helper, Mr. Christian Seifert. I am indebted to Messrs. T. W. Ludlow and Albert A. Stur, draftsmen in the office of the Superintendent of Buildings and Grounds of Columbia University, for the diagram (p. 409), and for help in making the description of the general construction of the cage (pp. 404–405).

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. No. 171.

THE STIMULATION OF THE INTEGUMENTARY NERVES OF FISHES BY LIGHT.

BY G. H. PARKER.

IN a paper published two years ago (Parker, :03), I described some experiments on the frog by which it was shown that, in confirmation of the results obtained by Korányi ('93), the integumentary nerves of this animal are sensitive to light, and that they enable it to orient itself to a source of light, even after the removal of its eyes. That the integumentary nerves in certain salamanders are also sensitive to light had already been demonstrated for Triton by Graber ('84) and for Proteus by Dubois ('90). Carlton (:03) has also shown a similar condition in a lizard, Anolis carolinensis; and it is likely from the observations of others that the integumentary nerves of many other lacertilians are sensitive to light. So far as fishes are concerned, I know of only one set of observations on sensitiveness of this kind; namely, those by Eigenmann (:00) on the blind fishes of the genera Chologaster and Amblyopsis, which were shown to be sensitive to light, presumably through the skin. No one, however, has tested this matter very fully for fishes, and it therefore seemed to me desirable to extend this line of work on these more primitive vertebrates. At my suggestion Mr. J. A. Long, while working at the Biological Laboratory of the United States Bureau of Fisheries at Wood's Hole, Mass., carried out a series of experiments with this object in view on the common killifish, Fundulus heteroclitus; but the individuals in which the optic nerves had been cut gave no conclusive evidence of reacting to light, and the experiments therefore led to a negative conclusion. It nevertheless seemed to me that, though the skin of Fundulus might not be sensitive to light, that of other fishes might well be; and this opinion

has been justified by the experiments on ammocœtes detailed in this paper.

The material on which I worked consisted of four living specimens of ammocœtes, each about 7 centimetres long. They were the larvæ of either the lake lamprey or the brook lamprey,[1] which had been sent me from Ithaca, N. Y., by Professor S. H. Gage of Cornell University. I wish to express here my indebtedness to Professor Gage for his kindness in procuring this material for me, as well as for having called my attention to this very satisfactory fish for experimental work.

Ammocœtes lives well in confinement, is easily etherized, stands operations well, and is sensitive to light.

My first experiments were directed toward ascertaining the character of the responses of the normal ammocœtes to light. These experiments were conducted for the most part in a dark-room with a single-glower Nernst lamp as a source of light. This lamp had in front of it a heat-screen consisting of a glass vessel with parallel sides 7 centimetres apart and containing water. Immediately behind the heat-screen was placed a small oblong glass aquarium at such a distance that the light intensity at its middle point was about 35 candle-metres. The base of this aquarium measured about 12 by 20 centimetres, and the aquarium was usually so placed that its narrow sides were at right angles to the direction of the rays of light. On the face of the aquarium next the lamp an opaque screen was so arranged that the light could be suddenly excluded from the aquarium or admitted to it.

When the four ammocœtes were introduced into the aquarium, the diaphragm having been so adjusted as to cut off direct light, they swam about for a while and finally came to rest on the bottom. After they had been at rest for some time, the opaque screen was quickly removed so that the full strength of the light fell on them, whereupon they all began swimming actively. This they continued to do till one by one they dropped to the bottom, there to rest a short time, and then to recommence swimming. It was noticeable that they swam almost always with the snout in one of the four corners of the aquarium, thus indicating a strong thigmotropism, as intimated by Gage ('93, p. 450). Their phototropism was also seen in these reactions, for they almost invariably swam into the corners of the aquarium farthest from the light. When one rose, after

[1] These species cannot well be separated at this stage (GAGE '93, p. 456).

having rested a certain time on the bottom, it turned almost without exception away from the light and swam toward the far end of the aquarium. Sometimes on rising the fish would strike the side of the aquarium in such a way as to become directed toward the light. If in such instances it soon reached a corner near the light, it remained there some little time, swimming with its snout close to the angle of the aquarium. Sooner or later, however, all such individuals made their way back to the rear of the aquarium. A quantitative statement of this condition can be made on the basis of the following counts, which give the numbers of individuals out of four in the half of the aquarium away from the light at half-minute intervals: 3, 3, 3, 3, 4, 4, 4, 4, 4, 3, 4, 4, 4, 4, 4; 3, 3, 4, 3, 2, 4, 4, 3, 4, 4, 4, 3, 4, 4, 3. The first fifteen of these records were taken with one end of the aquarium toward the light; as a check, the second fifteen were taken with this end away from the light. Had the ammocœtes been indifferent to light, they would have been found as often in one half of the aquarium as in the other; but, as the counts show, they were in the half of the aquarium away from the light 107 times out of a possible 120. It is evident from these records that ammocœtes is negatively phototropic, though its phototropism is at times overcome by its thigmotropism.

When the four ammocœtes were exposed to the light as just described, but were in a cylindrical aquarium instead of an oblong one, they swam round and round till they dropped to the bottom to rest. It was interesting to observe that as they swam past the sides of the aquarium nearest the source of light and farthest from it, they increased their speed noticeably; and they came to rest usually near the wall of the vessel and about midway between the side toward the light and that away from it. These reactions were undoubtedly determined by the intensity of the light, for near the periphery of a cylindrical vessel the regions nearest the light and farthest away from it are the ones of greatest illumination, and those between receive the least light. Ammocœtes is therefore not only phototropic, — *i. e.* the *direction* of its swimming is determined by light, — but it is also photodynamic, — *i. e.* the *rate* of its swimming is dependent upon light.

Having ascertained that the normal ammocœtes was negatively phototropic and photodynamic, I decided to find out next what parts of its body served as receptive organs in these reactions. The eyes in ammocœtes are well known to be somewhat degenerate, but since all the parts of the functional eye are present in this animal, it was

entirely possible that these organs might be of some real service in vision. To test the importance of the eyes for the light reactions just described, I prepared two ammocœtes by etherizing them, cutting the optic nerves, and allowing them twelve hours in which to recover. On testing these two animals in the oblong aquarium, I found them to react to light in essentially the same way as when they had their eyes intact. They were still strongly negative to light, and, under the same conditions of experimentation that were employed with the normal individuals, were found in the half of the aquarium away from the light 105 times out of a possible 120. When placed in the cylindrical vessel they also showed the same acceleration of rate in the more brightly illuminated portions as had been shown by the normal animals. In only one respect, so far as I could observe, did they differ from normal individuals: they were more easily caught by the hand. But whether this was due to some secondary effect of the operation or to the loss of the eyes directly, I am unable to say. Since in all other respects they acted like normal fishes, I am forced to conclude that the eyes in ammocœtes are not essential to its phototropic and photodynamic reactions. Unfortunately I was unable to devise an experiment to test the reactions of these animals to light reaching them exclusively through the eyes; therefore, though I am sure that the eyes are not essential to the animal's phototropic or photodynamic reactions, I am not in a position to deny that they take any part in these reactions.

I next tested the *skin* of ammocœtes for sensitiveness to light. For this purpose I placed the aquarium containing two normal ammocœtes in a shaded situation, but so near a window that by means of a small hand mirror I could easily throw a beam of sunlight on them. When a resting animal was thus suddenly illuminated, it would almost invariably start into active swimming. If the beam was made narrow and thrown on the anterior fourth of the animal's body only, including the eye, the response was always very slow in appearing and often entirely failed. Much the same was true when the beam was thrown on any portion of the middle half of the animal. When, however, it was thrown upon the tail, even though it illuminated only a small fraction of the caudal region, the animal almost immediately started into active swimming. From repeated trials of this kind, it was perfectly evident that the posterior end of ammocœtes was far more sensitive to light than any other part of the animal's body, even than that in which the eyes were situated. In-

deed, it was remarkable how quick and vigorous was the response when light was thrown on the tail in contrast with other parts of the body.

Reactions precisely similar to those just described were obtained from the two animals in which the optic nerves had been cut. One of these, even after it had been decapitated, still reacted to light. After the head had been removed, the animal no longer swam about the aquarium but remained resting on the bottom. When a beam of light was thrown on its middle portion, it seldom if ever responded, but when the light fell on its tail, it almost invariably made one or two locomotor movements. These reactions were so invariable that I have not the least doubt in ascribing them to the stimulating effect of light on the tail.

It might be maintained that the reactions of individuals in which the optic nerves had been severed or the head cut off were not due to the stimulation of the peripheral ends of spinal nerves by light, but to a direct action of light on organs in the spinal cord, such as is believed to occur in Amphioxus. But I am not inclined to accept this explanation, for ammocœtes reacts quickly when the light is thrown on its ventral side, a side from which the cord would be relatively inaccessible to light because of the thickness of body to be traversed. For this reason I believe that the light reactions of decapitated ammocœtes, or of those in which the optic nerves are cut, depend upon a stimulation of the peripheral ends of the spinal nerves in the integument.

It might also be contended that, at least in the case of the experiments with reflected sunbeams, the heat rays and not the light rays were the effective stimuli; but as most of these reactions took place after the rays had penetrated 8 or 10 cm. of water, this contention could have no weight, for such a depth of water would eliminate all the heat before the rays had reached the animal.

In order to get some idea of the intensity of light necessary to induce a reaction in ammocœtes, I threw a small beam of light directly from a single Nernst glower on the tail of a normal individual a few centimetres distant, but always without effect. I next tried light from a six-glower lamp, and found that when a beam from this source was thrown on the tail of a normal animal an immediate locomotor response followed, though the animal did not react to the same beam when thrown on the middle or anterior part of its body. By varying the distance it was found that the lowest intensity of light

that caused a reaction when thrown on the tail of the ammocœtes was about 20,000 to 25,000 candle-metres (a six-glower Nernst lamp at a distance of 12.5 cm. from the fish). No response was obtained when light of this intensity was thrown on the head or on the middle part of the body, and this may therefore be looked upon as the approximate threshold-intensity for the most sensitive part of the body of the fish.

From the observations thus far recorded I conclude that the spinal nerves of the integument of ammocœtes are sensitive to light, and that this sensitiveness is most marked at the tail. This condition is doubtless correlated with the well-known habit of ammocœtes to burrow. According to Gage ('93, p. 450), these fishes burrow head foremost into the sand far enough to hide themselves. Such a habit would be greatly facilitated by a tail sensitive to light, for by this means they would be kept burrowing till the tip of the tail was brought below the level of the sand in which they live.

The ability of the spinal-nerve terminals to be stimulated by light may now be said to be established for certain fishes, amphibians, and reptiles; and this fact is not without interest in connection with the theories of the origin of the vertebrate retina. Nearly twenty-five years ago Balfour ('81, p. 419) pointed out that the inversion of the vertebrate retina was most easily accounted for on the assumption that this layer had been derived from a light-receptive layer once functional on the outer surface of the ancestral vertebrate, and that in the formation of the central nervous system this outer layer had been inverted as it was swept into the deeper parts of the head. This view, which has been considerably elaborated recently by Boveri (:04), has received no small support from Hesse's discovery ('98) of retinal cells in the cord of Amphioxus; and the facts recorded in the present paper give it further support, since they indicate that even the surface of certain existing lower vertebrates still retains a sensitiveness to light like that which probably characterized the primitive external layer from which the paired eyes were derived. It must be kept in mind, however, that the terminal organs in the paired retinas are rod and cone cells and that even in Amphioxus the terminal organs in the cord are cell bodies, whereas in the skin of fishes, amphibians, and reptiles the terminal organs sensitive to light are probably free nerve terminations. This difference, however, indicates in my opinion a degeneration of the terminal organs in the skin of modern forms accompanied by a decline of the integument in

sensitiveness to light. It is possible, as I have elsewhere pointed out (Parker, :03, p. 34), that these degenerate light-terminals in the skin of the lower vertebrates become the temperature-terminals in the integument of the higher forms.

Summary.

1. Ammocœtes is negatively phototropic and photodynamic.

2. Its eyes are not essential in these reactions to light.

3. Its integument is sensitive to light, and this sensitiveness is greater in the tail than in any part of the trunk or head.

4. The terminal organs for light reception in the skin of ammocœtes are in all probability the free nerve terminations of the spinal nerves.

5. This sensitiveness of the vertebrate skin to light is probably a remnant of that primitive condition from which the lateral retinas were derived, and possibly served as a basis from which the temperature-terminals of the skin in the higher vertebrates developed.

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ON THE THEORY OF GEOTROPISM IN PARAMŒCIUM.

By E. P. LYON.

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THE general phenomenon of geotropism in the protozoa is known through the work of Schwarz,[1] Massart,[2] Jensen,[3] Sosnowski,[4] Miss Moore,[5] and others. If we place water containing paramœcia in a vertical glass tube, they tend to move upward and form a gathering near the top. The reaction is more prompt and exact in some cultures than in others. It is easily disturbed by food or other chemical stimuli, by contact relations, by change of temperature, etc. For this reason it is best for geotropic experiments to have glassware very clean and to place the animals in very pure distilled water. Under such conditions the response seldom fails with active, well-fed animals. That gravity is the cause of the orientation and gathering is shown in two ways. First, by exclusion methods it can be demonstrated that neither light nor the downward diffusion of oxygen, nor any other condition except gravity, is the cause of the phenomenon. Secondly, by subjecting the animals to a properly graduated centrifugal force it can be shown that they go against an accelerating force of the same character as gravity. We therefore know that paramœcia under usual conditions are negatively geotropic.

When we come to ask how gravity is able to stimulate the organisms, or (using an anthropomorphism) how they "know up from down," we find that several views have been advanced. We will consider each of these in turn.

Verworn[6] in his early work was unable to see how gravity could be

[1] SCHWARZ: Berichte der botanischen Gesellschaft, 1884, ii, p. 51.

[2] MASSART: Bulletin de l'Académie royale de médecine de Belgique, 1891, xxii, p. 158.

[3] JENSEN: Archiv für die gesammte Physiologie, 1893, liii, p. 428.

[4] SOSNOWSKI: Bulletin de l'Académie des sciences de Cracovie, 1899.

[5] MOORE: This journal, 1903, ix, p. 238.

[6] VERWORN: Psycho-physiologische Protisten-Studien, 1889, Jena, p. 122; reference from Davenport's Experimental morphology, i, p. 121.

regarded as a stimulus. Acting equally all the time, it seemed to him quite different from an induction shock, for example, which is applied for a fraction of a second only. Regarding this view it may be said that if it should seem best to restrict the word "stimulus" to sudden changes of relation between environment and organism, it must still be borne in mind that such change of relation may arise from movement of the animal just as well as from movement of the environment. I have emphasized this point in my paper on Rheotropism.[1] In the case of gravity we have, it is true, a force unchanging in direction or magnitude; but the movement of the animal brings it from time to time into new relations with this force, and the conditions of stimulation are therefore fulfilled. We know that an electric current flowing across a nerve does not stimulate; flowing lengthwise, it does. Stimulation would result if the nerve were moved from the crosswise to the lengthwise position on the electrodes, just as surely as if the electrodes were moved from one position to the other and the nerve remained at rest. So we might assume that the lines of the force of gravity do not stimulate when they pass lengthwise through a paramœcium, but do stimulate when they cut across the organism. Stimulation would occur whenever the organism came into a horizontal position, just as surely as if gravity were a force to be moved about and applied at will. This illustration is introduced, not as a picture of what actually takes place, but for the purpose of showing that gravity possesses all the qualities of a stimulus, even in a restricted sense of that word. Jennings[2] has emphasized the same ideas regarding chemical stimuli: "Stationary as the stimuli may be, the organisms . . . are *not* stationary; by their own movements the organisms come into new relations with the stimuli," etc.

The misconception of gravity as a stimulus into which Verworn fell, led him to advocate what has been called the *mechanical* theory of geotropism. It was supposed that the protozoa took up positions in the water determined by the laws of buoyancy, — the positions, in other words, which, as dead bodies of the same density and shape, they would take. In paramœcium, for example, if the centre of buoyancy were nearer the anterior end than the centre of gravity, the animal would stand upright in water for the same reason that a ship does, or a hydrometer. That the assumption is incorrect was indicated by Jensen when he found that dead euglena fell with the

[1] Lyon: This journal, 1904, xii, p. 149.

[2] Jennings: American naturalist, 1900, xxxiv, p. 264.

anterior end directed downward, while the living forms place the anterior end upward, move upward, and are negatively geotropic. The only criticism of Jensen's experiment is that it did not exclude the possibility that in killing the animals the centres of buoyancy and gravity might have been changed through distortion or through localized changes of density in the protoplasm. It is true that he used those killing reagents which produced least visible distortion, and the probable correctness of his conclusion has been generally accepted. But inasmuch as his method was not entirely above criticism and the proof had not been extended to paramœcia, I looked for a method that could be applied to the living, unaltered organisms. I made use of the fact that the animals when centrifuged strongly are passively thrown to the bottom of the tube. A glass tube was drawn into a capillary so fine that paramœcia could not turn around in it (see Fig. 1). The capillary end *C* was dipped into distilled water and the tube filled by capillarity to point *A*; then the end *C* was sealed and a drop of water containing paramœcia was introduced into the large part of the tube. The tube was fastened to the centrifuge so that the capillary pointed away from the axis of the machine. After strong rotation the capillary was examined under the microscope. The organisms were invariably found with anterior ends directed towards the closed outer end of the tube. It is therefore certain that passive paramœcia would fall head end down, and that their negative geotropism is the result of an active process on the part of the animals. Jensen's view in this respect is fully confirmed, and the mechanical theory is to be laid entirely aside.

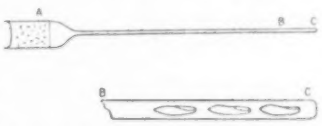

FIGURE 1. — Tube ready for centrifuge, and a part of capillary after centrifuging, magnified, showing paramœcia forced down head first.

The theory of Jensen,[1] adopted by Verworn in his text-book of general physiology in place of his own original hypothesis, may be called the *pressure* theory. Jensen supposes that the organism responds to hydrostatic pressure and tends to move from places of high to those of low pressure. If we consider the conditions necessary for stimulation, it is apparent that the difference in pressure between the upper and lower surfaces of the animal must be the stimulus to orientation, because, if the pressure were exactly equal on each element of surface, it is impossible that the animal should

[1] JENSEN: *Loc. cit.*

"know up from down," no matter how great the pressure. This difference in pressure between the two ends of the animal is constant. If we calculate what ratio it sustains to the total pressure, we find (as Jennings [1] and I [2] have shown independently) that in the case of paramœcium it is less than one-millionth of the pressure on one surface of the animal. This supposes a sensitiveness to mechanical stimulation which Jennings has shown the paramœcium does not possess. If we imagine a man able to appreciate the direction of the vertical by the excess of atmospheric pressure on his feet over that on his head, we begin to see the improbability of Jensen's theory. Indeed, the pressure on a person's feet is more than two hundred millionths greater than that on his head.

From considerations such as the foregoing I came seriously to doubt Jensen's hypothesis, and determined to examine the experimental evidence for and against it. Jensen cites an experiment in which the tube containing the organisms was inclined to the vertical. In such a tube paramœcia often move vertically upward until they strike the upper wall of the tube, and then follow the direction of this wall to the surface of the water. This proves, he thinks, that the animals are oriented in the line of pressure rather than in that of gravity directly. The only possible basis of this argument seems to have been the belief that the pressure varies in the direction of slant of the tube. In reality, of course, the pressure varies with the vertical depth, and is independent of the slant of the tube.

A second line of experimentation used by Jensen was the artificial variation of the pressure. If the paramœcia were placed under the receiver of an air pump and the air exhausted, the hydrostatic pressure was greatly diminished. The difference in pressure between the upper and lower surfaces being constant, the ratio between the supposed effective or stimulating pressure and the total pressure is greatly increased; and we might expect a more definite orientation. Jensen claims, indeed, that the geotropic response is strengthened.

The experiment is subject to the criticism that, on pumping away the air from above the tube, the oxygen and other gases leave the culture medium. This affects the activity, and quite likely the accuracy of orientation, of the animals. I repeated the experiment in the following way: Two tubes of water containing paramœcia were freed from air, and a layer of oil placed on the water in each. One

[1] JENNINGS: Journal of comparative neurology, 1904, xiv, p. 441.
[2] LYON: This journal, 1905, xiii, p. xv.

tube was then connected with a vacuum pump, and the air pressure reduced to a few millimetres of mercury. No hastening of the geotropic gathering was noticeable in this tube. The contrary, rather, was true.

Jensen studied the effect of increased pressure, on the supposition that he might be able to reverse the geotropism thereby. This he was unable to do. I tried the same experiment with a different end in view. Since the pressure effective as a stimulus according to Jensen's theory must be constant, by increasing the total pressure the definiteness of orientation should decrease; and one might reach a point at length where the pressure differential would be too small, and geotropism would fail. No such failure was noted on increasing the pressure to three atmospheres, although the animal must then be supposed to be responding to a stimulus of less than one three-millionth of the pressure on one side of its body, or to a much smaller fraction of the total pressure.

However, in my opinion, little weight, one way or the other, should be given to experiments involving great changes of pressure. The internal condition and general activities of the organisms are undoubtedly so changed that the experiments have little significance as bearing on the theory of geotropism.

Another observation of Jensen's is, however, of very great importance, and gives the clue to a correct solution of the problem. If paramœcia are observed immediately after being moderately centrifuged, their geotropism is found to be much more pronounced than normally. Jensen interpreted this as due to increased pressure. But this cannot be true. If we calculate the increase of pressure it is found to be very small, compared with the increase in centrifugal force over ordinary gravity. Assuming the tube to be 10 cm. deep, its open end to be at the axis, and the rotation sufficient to cause a force of 10 g at the bottom of the tube, the increase in pressure at the bottom is only 4 per cent, while the increase in g is 900 per cent. If we place only 1 cm. depth of water in the tube, the pressure is increased less than 1 per cent by a centrifugal force of 10 g. But in 1 cm. of water the geotropism is as much reinforced as in 10 cm. Moreover, increases of pressure produced in other ways do not strengthen geotropism. Finally, a variation of Jensen's experiment proves beyond all doubt that his theory is wrong. A tube closed at one end and nearly closed at the other is filled with water containing paramœcia. It is arranged on the centrifuge so that its *open end is away from the*

axis. By careful rotation the animals are driven to the outer open end of the tube without being forced out.[1] Now, the protozoa so handled show afterward the same pronounced geotropism that Jensen described. But the pressure at the open end is never greater than atmospheric. Here we have the crucial experiment. Pressure is not raised, but the force equivalent to gravity is increased. The geotropic response is strengthened. Therefore geotropism is directly dependent on gravity and not on pressure. We may safely discard the pressure theory of geotropism.

A third theory of geotropism is based upon the assumption that the animal meets a greater resistance in moving upward than in moving downward. We may call this the *resistance* or *weight* theory. As Davenport[2] puts it, in swimming up, the animal must overcome friction plus weight; in swimming down, friction minus weight. The animals are supposed to be impelled to travel against the greater resistance. While this theory seems easily assailable on physical grounds, involving on final analysis the assumption that paramœcium can appreciate differences of velocity of movement, both active and passive, it is perhaps sufficient to bring forward experimental evidence against it. This is found in the successful application of the method suggested by Davenport and used by Miss Platt.[3] The organisms were immersed in a liquid of the same density as themselves, and definite geotropic gatherings observed. As they have no weight in such a liquid, the supposed basis of stimulation falls away. Miss Platt tried the experiment, but was unable to find a strongly geotropic culture. Miss Moore[4] states that she succeeded with it, and I was in doubt about her result only because the specific gravity of the organisms was so imperfectly known. Of course, this is a necessary prerequisite. Jensen attempted to find it by the very bad method of immersing the animals in potassium carbonate. Miss Platt attempted to ascertain the density of spirostomum, paramœcium, and tadpoles by measuring the density of that solution of gum-arabic in which dead or anæsthetized organisms would neither rise nor fall. The experiment is subject to two criticisms. First, the density of the

[1] I find that a variation of this experiment described in this journal, 1905, xiii, p. xv, is not susceptible of the interpretation there advanced. Pressure does not increase toward the axis.

[2] DAVENPORT: Experimental morphology, 1897, i, p. 122.

[3] PLATT: American naturalist, 1899, xxxiii, p 31.

[4] MOORE: *Loc. cit.*

animals is very likely to be changed by any killing reagent or anæsthetic; second, the viscosity of such a solution is such that even bodies of considerably different density are likely to be held up for a long time unless a greater force than gravity be used to separate them from the solution. The first criticism is extremely important, and I therefore looked for a method by which the density of small organisms could be found without any injury or change in them. I made use of the hæmatocrit attachment of the centrifuge by which very high velocities of rotation and consequent centrifugal force are secured. At the bottom of a small tube was placed a little gum-arabic solution of known density; above this a few drops of water containing paramœcia. The tube was then centrifuged about one minute. If the animals were forced to the bottom of the tube, their density was known to be greater than that of the gum solution used. By varying the latter the density of the animals is easily found. I should add that centrifugalizing for the above length of time produces no effect in the general appearance nor in the activity of the organisms.

Here are the protocols of such an experiment:

CULTURE 5.

I. { Tube 1. Density of gum sol. 1,050, 12,000 revolutions; a few go to bottom.
Tube 2. " " " " 1,052, " " ; very few go to "

II. { Tube 1. Density of gum sol. 1,048, 10,000 revolutions; over 50 % go to bottom.
Tube 2. " " " " 1,052, " " ; a few " " "

III. { Tube 1. Density of gum sol. 1,043, 10,000 revolutions; all go to bottom.
Tube 2. " " " " 1,048, " " ; greater part go to bottom.

This and other experiments show that the density of paramœcia may vary from about 1,042 to 1,054. Those in distilled water may be slightly less dense than those in the culture medium. An average density of paramœcia is about 1,048 or 1,049.

The figure obtained by Miss Platt, 1,017, is so different that I tried a culture at Woods Hole in addition to four or five in St. Louis. The results vary only within narrow limits. The method is applicable to any small organisms, and I shall use it for further studies. I should, perhaps, add that the gum solutions used were the same as those about to be described.

The solutions used for the geotropism experiments were made as follows. Only water which had been double distilled in glass was used. In this water paramœcia will live over a week. Powdered gum-arabic was dissolved in this water so as to form a strong solu-

tion. The slight acidity always found was then very carefully neutralized with sodium hydroxide. Then the solution was dialyzed against distilled water for several days. In solutions[1] so prepared, even when considerably denser than the organisms, the latter will live a week or more. From the original solution several dilutions were made, and the densities of all ascertained by the pycnometer. Intermediate densities were secured by mixing any two of the stock solutions in proper proportions.

The further procedure was very simple. A drop of distilled water containing many paramœcia was thoroughly mixed with the gum-arabic solution and placed in a tube. A layer of paraffine oil was placed on top of the liquid. This was intended to exclude oxygen and a possible consequent chemotropic gathering. Invariably in all the solutions, whether of equal, greater, or less density than the animals, the latter slowly rose and formed a dense ring near the top. The response was slow, as the velocity of swimming in such viscous solutions is extremely small. Two or three hours, or over night was often necessary. But often the rising ring of animals could be seen in twenty minutes or less. It was typical geotropism very much slowed.

The only criticism of this experiment, which I see, is that the osmotic pressure of the gum is not entirely negligible, as the density of the animals is somewhat increased by a prolonged stay in such solutions. During the stay of about one day in gum of density 1,049, paramœcia of originally the same density attained a density of about 1,054. In another case, paramœcia of density 1,049 were increased to 1,053 by a stay of twenty hours in gum of density 1,051. But these changes are so slight, and the resistance introduced by the thick gum so very much greater, that the resistance theory is rendered more than improbable. Moreover, as Miss Platt points out, if geotropism were a weight effect, "the negatively geotropic organism should become positively geotropic in solutions of greater specific gravity than its own." Such is never the case. The organisms, indeed, come quicker to the top in solutions of greater density than their own than in those of equal density, being buoyed up to so great an extent that the increased viscosity is more than balanced.

[1] I cannot too highly recommend this solution for the examination of paramœcia or other motile unicellular forms. Teachers of zoölogy will find it far superior to cotton, gelatin, or any other device for holding paramœcia still. The careful neutralizing is very essential.

Having shown that gravity produces orientation neither through pressure nor weight, we are driven to the idea that it acts on the cell directly; that the internal constitution is affected and determines the response. This theory has always appealed to the writer for two reasons. First, and most important, it is the only one that can be applied to the majority of cases of geotropism in plants and in hydroids. Secondly, in my studies on compensatory motions[1] I found traces of geotropism in crustaceans after removal of their specific geotropic sense organs and blackening of the eyes. This residuum of geotropic response I was inclined, without any experimental evidence however, to attribute directly to cells of the body, *e. g.*, of the nervous system. It seemed reasonable that certain cells might retain some degree of direct irritability to gravity, provided that unicellular organisms had such a quality.

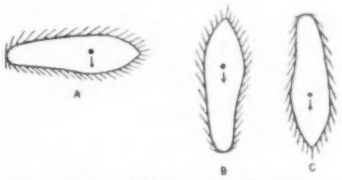

FIGURE 2. — Scheme of stimulation, Statolith theory. See text.

When we consider what must be the internal condition in order that gravity may influence a cell directly, we see at once that differences of density within the cell must be predicated. For internal stimulation the relation of the parts of the cell to each other must be changed in some way by gravity. Stresses or pulls which occur when the organism is in one position with respect to the vertical, must be changed in another position. This could not take place in a homogeneous body, nor in a complex one all of whose elements were of equal density. But if some are of greater density than others, such pressures and pulls must occur, and can easily be supposed to produce the effects which we have seen. In Figure 2 this is illustrated. The heavier particle (which, of course, is not necessarily the nucleus) must, in position *A*, push against the lower and pull upon the upper side of the organism. In position *B* it must pull upon the posterior end and press against the anterior. The reverse of *B* holds true in position *C*. If the particle were lighter than the rest of the protoplasm, similar but opposite inequalities of stress would result, and the theory would not be changed. Now, it is evident that for the animal in position *A*, it is sufficient to imagine a slight inhibition of cilia produced on the upper, or a slight stimulation on the lower side, and we should have the orientation sufficiently explained according to the ordinary

[1] LYON: This journal, 1899, iii, p. 86.

tropism schema. Or if we imagine that the inequalities of pressure in positions *A* and *B* lead to the "avoiding reaction," while those of position *C* do not, we should be able to account for orientation on the trial and error method, which according to Jennings[1] (and I have studied the response sufficiently to believe that he is entirely correct) is the actual method of orientation to gravity in paramœcium. The spiral locomotion would make no difference provided stimulation take place in positions *A* and *B*. According to Jennings[2] this organism occasionally makes the avoiding reaction when no external cause can be detected; in all probability, therefore, in response to internal changes. We have, then, only to imagine the changes produced by gravity to be somewhat of the nature here outlined to have a fair picture of geotropism in these forms.

It is well known that the substances within cells are not always of the same density. Herrick[3] showed that the nucleoli of the ovarian eggs of the lobster are always at the lower edge of the nuclei. It was interesting to see whether it could be demonstrated that the paramœcium also is actually composed of substances of various densities. The animals were strongly centrifuged for several minutes in the haematocrit attachment. Microscopic examination showed that certain dark granules originally distributed were now aggregated in one end, usually the anterior. It is thus seen that differences in specific gravity exist in the protoplasm of this animal.[4] Moreover, it is to be observed that paramœcia which have been very strongly centrifuged have lost their geotropism for a time, although still active. Indeed, strong centrifugalization with its accompanying displacement of substance is quite different in its effect from moderate centrifugalizing, which, as has been said, intensifies the geotropism. In the latter case we can imagine the stronger pull of the denser substance to be followed by stronger stimulation. In the former the whole system is upset by actual displacement of the heavy (or light) substance.

[1] JENNINGS: Journal of comparative neurology, 1904, xiv, p. 441.

[2] Personally communicated. I have also noticed this phenomenon.

[3] HERRICK: Anatomischer Anzeiger, 1895, x, p. 428.

[4] Later experiments show that the eggs of many animals can be separated into a number of substances which arrange themselves in perfectly distinct zones. In the Arbacia egg four such substances can be made out. Taken in connection with the recent work of Conklin and others on organ-forming substances, these observations possess considerable interest. I am carrying on further investigations along this line. The nucleus, it may be added, is found to be lighter than the cytoplasm in all cases investigated. The usual description of the nucleus as "denser than the cytoplasm" is to be understood as referring to optical density only.

It will be seen that the above theory of geotropism is of the same character as the statocyst (otocyst) theory in higher animals. There we have a complicated sense organ depending for its action on the pull or pressure of a denser structure; for example, the statolith (otolith). It has been shown by Clark[1] that an actual stone is not necessary; but in the Brachyura studied by him and which have no otoliths, one would undoubtedly find that certain parts of the otocystic structure are of greater specific gravity than others, and thus the conditions for stimulation provided.

The botanists are divided as to the cause of the geotropic curvations which are so common in plants. But the statocyst theory receives a large acceptation. Such bodies as starch grains and oil drops are supposed to be equivalent to statoliths and bring about gravity stimulation. A large amount of evidence in this direction has been brought forward by Haberlandt[2] and others. If in paramœcium geotropic stimulation were attributed to food particles or to storage materials, we should have an explanation of the failure of geotropism in starved animals, which is sometimes very marked. The case would be exactly parallel to that of rootlets, which are said not to show geotropism when all their starch has been exhausted.

Summary.

Experimental. — 1. Living paramœcia are precipitated by the centrifuge anterior end foremost. Therefore the geotropic orientation is an active process. The *mechanical* theory for this reason cannot be true.

2. A variety of experiments show that Jensen's *pressure* theory is incorrect, especially the fact that geotropism is intensified in a centrifuge tube which is open away from the axis and in which, therefore, no increase of pressure above atmospheric is possible.

3. The *resistance* or *weight* theory must be given up because paramœcia are geotropic in solutions of the same density as themselves, and in which, therefore, the resistance is the same going up as down.

4. The specific gravity of living paramœcia was determined to be about 1.049.

[1] Clark: Journal of physiology, 1896, xix., p. 327.

[2] Haberlandt: Jahrbücher für wissenschaftliche Botanik, 1903, xxxviii, p. 447.

Theoretical.— It seems that gravity must act directly upon the inner constitution of the cell. This must involve pressures or stresses within, which could only come about in a system of substances of different densities. It can be shown that such differences of specific gravity actually exist in the body of paramœcium. The theory which explains the facts best is therefore practically the same as the statocyst theory developed for higher animals, except that instead of a complicated sense organ and reflexes, we have here the whole mechanism of stimulation and response in a single cell.

RESTORERS OF THE CARDIAC RHYTHM.

By D. J. LINGLE.

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Introduction.

MODERN physiology teaches that certain methods of treatment will make living but non-beating heart tissue begin to beat again. This treatment consists in the application of electrical, mechanical, and chemical agents. Of these the electrical include the constant current and induction shocks, both slowly repeated and tetanizing. The mechanical includes pressure in the heart's cavities, tension in suspended heart strips, and the various forms of mechanical injury, as pricking and so forth. The chemical members belonging in this class are numerous, and include a large number of substances completely devoid of chemical relationship. The idea that these agents possess rhythm-restoring powers is based on the results of experiments carried out under widely differing conditions and with material of different kinds. Some of the work has been done on entire hearts, both excised and in the body, some on heart strips, and both vetebrate and invertebrate hearts have been used. But whenever a test has been made of the powers of an agent to restore a lost rhythm with whatever kind of material, the method used has almost invariably been such that no distinction could be made between the action of the agent being tested and that of the solution of sodium chloride invariably used with it. This error in method resulted from the prevalent idea that a solution of sodium chloride was neutral and played no part in the production of a restored rhythm.

We now know that this idea is not true, and we can legitimately doubt conclusions from every experiment of this kind where precautions were not taken to exclude the effects of the sodium chloride solution. The results contained in this paper have been secured by a study of the powers of some of the agents commonly considered able to restore a cardiac rhythm under conditions that exclude the

assistance of a solution of sodium chloride, either in the form of a bathing solution or moistener.

Each agent tested has been made to exert its powers on the same kind of tissue, *i. e.*, a strip from the ventricle of a turtle's heart, and under the same conditions. It was expected this would reveal the comparative value of each kind. But the results do not, and, as will be shown, they indicate that under the conditions of experimentation many so-called rhythm restorers do not have this power, *per se*, at all. This holds true for electrical and mechanical conditions, and certainly for some chemical agents also. The electrical and mechanical agents have been especially worked with, and it is found that their rhythm-restoring power is entirely lacking when used independently of a solution of sodium chloride.

These results reveal from a new standpoint the value of sodium chloride in the phenomena of heart physiology, and if they apply universally will simplify that subject by excluding from the field of discussion a number of agencies whose explanation would make more difficult the final solution of the problem of the origin of the heart rhythm.

Electric Currents as Rhythm Producers or Restorers.

I. The constant current. *A. Action of the constant current on strips from the ventricle of the turtle.* — Of all the so-called rhythm producers the constant current apparently occupies the securest position. Its claims for insertion in this class were first put forth by Eckhard,[1] and since his time they have been supported by a formidable literature. The conclusion of all this work may be summed up in the words of Biedermann,[2] who states that "the constant current during its closure in cardiac muscle thus produces a regular and invariable series of rhythmic contractions, which also appear, at least under certain conditions, in striated skeletal muscle; and this is still more the case in smooth muscle." Or we may quote Langendorff,[3] who states that "the apex of a frog's heart beats rhythmically in response to a constant current, and its rate varies with the strength of current used." Kaiser[4] has attempted to show that this power is due to

[1] Eckhard: Beiträge zur Anatomie und Physiologie, 1858, i, p. 145.
[2] Biedermann: Electro-physiology (English translation), 1896, p. 197.
[3] Langendorff: Archiv für die gesammte Physiologie, 1895, lxi, p. 336.
[4] Kaiser: Zeitschrift für Biologie, 1894, xxx, p. 279; *idem*, 1895, xxxii, p. 464.

rhythmic variations in the strength of the current used, and so is not really a constant current effect as it is usually understood. But Trendelenburg [1] has combated this idea, and with others regards the constant current as a real rhythm producer.

A review of this literature shows all of these observers made experiments on heart tissue either kept in or moistened with a solution of sodium chloride while under the influence of the constant current. As the solution of sodium chloride alone will start a rhythm, conclusions based on such experiments are of questionable value, especially when it can be shown that if the tissue from a turtle's ventricle is subjected to a constant current without the solution of sodium chloride no rhythm results.

The method used to test the action of a constant current *per se* on strips of a turtle's ventricle was as follows. Two heart strips were prepared from the apical region of the ventricle in such a way that a ring-like band with its long axis parallel to the base was removed. This was cut so that two pieces were secured as near alike as possible. One of these was suspended in a moist chamber and fastened to a lever. The other strip, also fastened to a similar lever, was kept in the air. The levers of both preparations were arranged to record on the same drum one over the other, and the drum usually made one revolution in twenty-four hours. These strips were so wired that a constant current would enter one strip, leave it, and enter the second. In this way both strips were traversed by the same current. The current used was our laboratory house current cut down to proper strengths by means of lamps and graphite rheostats. The feeblest current used was one that when interrupted while flowing through a frog's sciatic caused a minimal contraction of the leg muscles. Next above this, one was used that caused a maximal contraction. Then one kept just below a strength that would cause a contraction in the heart strip, followed next by one that always caused a good strip contraction. Beyond this the current was measured with a milliammeter and strengths of one-quarter, one-half, three-quarters, one, two, three, and more milliampères were used. It was easy, when working with strong currents, to know when an excessive strength was reached, for a too strong current threw the strip into a state of strong tonic contraction, — a condition which always interfered with its subsequent use.

Non-polarizable electrodes were used in all the experiments. Of

[1] TRENDELENBURG: Archiv für die gesammte Physiologie, 1900, lxxxii, p. 268.

these several kinds were employed, — Porter's boot electrodes, Ostwald's calomel electrodes, and the ordinary kaolin and zinc sulphate form. While experimenting, the strip in the moist chamber was kept moist by surrounding it with wet cotton so arranged as not to exclude air. The control strip in air was moistened with a solution of sodium chloride which was dropped on it or applied with a brush. In the first series of experiments boot electrodes moistened with a sodium chloride solution were used on both strips. And it was found that with all strengths of current the lower strip kept in air and moistened with the sodium chloride solution never failed to develop a rhythm, while the comparison strip in the moist chamber, receiving the same constant current but no sodium chloride, seldom did. When a rhythm did appear, it was feeble and imperfect. These strips were, however, in good physiological condition, for whenever the lower strip was moistened the other contracted rigorously, showing its irritability was sufficient to respond to the variation in the strength of the traversing current produced by the drop of sodium chloride solution on the other strip changing its electrical resistance.

But we had here to contend with the fact that sometimes a rhythm, feeble and imperfect it is true, did appear in the strip not moistened with the solution of sodium chloride. To see if this was due to the small amount of sodium chloride in the electrodes, others were substituted moistened with a solution of lithium chloride. And with such electrodes the strips in the moist chamber never developed rhythmic activity with any strength of current, while the control moistened with sodium chloride never failed. The same result was secured where all four electrodes were soaked in the lithium solution. Hence the effect cannot be due to an inhibitory action of the latter. In these experiments the preparations stood twenty-four hours, and ample time was given for activity to appear. It is not claimed the conditions remained the same for the whole period. They certainly did not; some electrodes were polarized in two hours, others lasted longer; but, nevertheless, it is true that while the favorable conditions lasted, the sodium chloride treated strip developed a good rhythm and the other strip did not, though the same constant current passed through both strips during the entire experiment.

Experiments of this kind indicate clearly the relative values of the sodium chloride solution and the constant current. When a strip is moistened with the solution and traversed by the constant current, its rhythm is renewed. But the constant current is not the active agent,

for the sodium chloride alone renews the rhythm, and the constant current itself fails to restore the rhythm when conditions are the same and the sodium chloride bath is omitted. Sometimes when working with strong currents, from three-quarters up to three milliampères, a strip moistened with the sodium chloride solution and traversed by the current will beat with a regular but very feeble rhythm. This condition was seen in winter, and was first attributed to a poor condition of the heart tissue. This, however, was not the cause, as careful inspection showed that the feeble rhythm was due to only a part of the strip beating rhythmically. All activity in the tissue at such times was confined to the anode end of the strip; the cathode region showed no signs of a rhythm. And the force of the strip's beats seemed feeble because only a part of the strip was working, and working with possibly diminished force when the whole strip was short. If a very long strip was used, then activity about the anode was quite as strong as in a piece of the same length when treated with a pure sodium chloride solution. Under these conditions the strip clearly showed what workers with the constant current have called polar activity. And as the result was the opposite of some phases of the law of polar action, I was careful to repeat the experiment a number of times, and there is no doubt that with a certain strength of current the rhythm in a heart strip is limited to the region of the anode. It begins there, and will disappear or greatly diminish when the anode is changed to a cathode. In experiments of this kind large hearts were used and extra long strips secured, and they were usually clamped in the middle with a Gaskell clamp, though sometimes they were simply ligatured firmly and suspended so that each half recorded on the same drum. Non-polarizable electrodes of several kinds were used to make sure they were neutral in the matter, and the results were the same with all kinds. Under these conditions, when one end was anode and the other cathode, activity of one or the other end was limited to about half of the strip. When a strip prepared in this way was traversed by a current of from three-fourths to three milliampères and moistened as needed with a solution of sodium chloride, it usually showed at first a number of simultaneous single contractions in each half. These were clearly produced by sudden variations in the strength of the traversing current, as they corresponded exactly with each application of the salt solution. After a time, though, it was found that the part of the strip in contact with the anode began to beat with a normal rhythm, while the cathode part remained absolutely without

such activity, though it continued to show strong individual beats when the strip was moistened. If under these conditions the direction of the current was changed, then the rhythm of the previous anode end began to diminish gradually, and a rhythm started in the new anodic region. Sometimes the rhythm in the former anodic space ceased when it was made cathodic. At other times it was depressed to microscopic proportions. In one experiment the current was reversed six times, and in each case the rhythm entirely ceased in the cathodic area and began again in each new anodic space. In another case reversal of the current worked the same with three reversals.

While these rhythms were active the strips showed a marked loss of tone. And the tone diminished equally in both anodic and cathodic regions, where there was activity as well as where it was lacking, thus showing that loss of tone and rhythm are independent, and causes that produce the one do not necessarily bring out the other.

This idea that rhythm is associated with anodic conditions has been recorded by several observers. Bernstein,[1] working with the constant current on a frog's heart, noticed that "in general the contractions run in the direction of the current and pass from the positive to the negative pole." His explanation was based on the idea that the heart's ganglia possess exciting and inhibitory powers and the direction of the current determines which power shall be active. But in a heart strip, Bidder's ganglia, the ones Bernstein evidently considered important are lacking, and such an explanation does not hold. Neumann[2] observed the same thing in the frog's heart. Schillbach[3] saw something similar in the intestine of the rabbit, where a single contraction originated at the cathode and a series of peristaltic contractions (a sort of rhythm) at the anode when it was acted upon by the constant current.

The literature of the subject contains some material that may be used to explain these observations.

First, there is the explanation of Biedermann,[4] who maintains that all these cases are simply cathodic effects. The conditions, he thinks, are such that physiological cathodes are formed, and he believes

[1] BERNSTEIN: Untersuchungen über den Erregungsvorgang im Nerven und Muskelsystem, Heidelberg, 1871, pp. 216 and 224.

[2] RICH. NEUMANN: Archiv für die gesammte Physiologie, 1886, xxxix, p. 408.

[3] SCHILLBACH: Virchow's Archiv, 1887, cix, p. 284.

[4] BIEDERMANN: *Loc. cit.*, p. 254.

we are in reality dealing with a phase of the law of polar stimulation. So far as I can see, he has no evidence to prove this, and it seems as though he was led to advance the idea in order to bring the phenomena under the law of polar stimulation. Even granting that it does, this is no explanation. We know very little about the actual working of electricity on animal tissue. Besides, the idea is unsatisfactory from another standpoint. It assumes rhythm-producing agents and contraction-producing agents are identical, but we have some evidence that these are more or less distinct. Heart muscle ordinarily shows rhythm and contractility, but it can easily pass to a state where it is still powerfully contractile but not at all rhythmic. In this condition it obeys the law of polar stimulation, but it by no means follows because heart muscle contracts at the cathode when a current is made through it that a rhythm must also be set up there. To maintain this is to hold the law applies in another field.

It is possible to disprove experimentally this idea when a constant current is made to pass through a heart strip of short interlacing fibres. Either a single anode and cathode is produced, or a whole series of anodes and cathodes. If the former is true, the idea is overthrown. If the latter represents conditions, then Engelmann[1] has shown that the efficiency of a pole is due to current density about it. Hence it must be the current density about the anode that produces the effect. And yet by changing the shape of the electrode contact we can completely change electrical density there without influencing the results. If we use an electrode with a surface of contact of greater area than the cross section of the strip, current density is not the same as when one of small area is used, and yet the result is unchanged. The phenomenon is entirely independent of the shape and kind of electrode, and therefore of current density also, and it is difficult to see how we can be dealing with a physiological cathode. Herring[2] has insisted that secondary anodes and cathodes are produced by bending or pinching muscle fibres, and our method of using Gaskell's clamp certainly falls in this class. But here, again, we have evidence that pinching has nothing to do with results, for, as has already been shown, the same thing is seen in unclamped strips. In these the rhythm can be seen in the part of the strip about the anode only, even when no mechanical separation of any kind exists between the anode and cathode.

[1] ENGELMANN: Archiv für die gesammte Physiologie, 1870, iii, p. 254.

[2] HERRING: Sitzungsberichte der Wiener Akademie, 1879, lxxxvii, p. 241.

Second, this anodic rhythm may belong in the same class with an obscure phenomenon first observed in muscle by Kühne[1] and called by him the "Porretsche Phänomen." By others it has been called the "Kühneschen Phänomens" and the "Galvanische Wogen." This consists of a series of wave-like contractions that begin at the anode and run over a muscle when it is traversed by a constant current of proper strength. Hermann[2] has studied this rhythm, and he states that it occurs only in parallel fibred muscles. If this is true, it is distinct in character from the rhythm seen in a strip of heart muscle. Nevertheless, I am strongly inclined to believe that this phenomenon, which has never been satisfactorily explained, is a rhythm of identical character with the anodic heart rhythm and has the same explanation, which will be given later.

We find, then, that under the influence of the constant current heart and possibly other, tissues develop rhythmic activity in the region of the anode; and this has always been considered a result of the action of the current at that pole. But we may now, it seems to me, ask the question: Is the phenomenon really a polar effect? If the fundamental idea of this paper is correct, this apparently anodic or polar rhythm is nothing more or less than a sodium chloride produced rhythm. It must be remembered that all experiments showing this phenomenon are made on tissues moistened with a solution of sodium chloride. And if we suspend a heart strip and moisten it with a solution of sodium chloride, after a time the whole strip shows rhythmic activity. If now we suspend and moisten in the same way a similar strip, and send a constant current of proper strength through it, after a time it shows rhythmic activity about the anode. This cannot be an anode effect, because the production and maintenance of an anodic state has no such power when the sodium chloride bathing solution is lacking. Beside, if the current is lacking and the moistening solution present, not only the part about where the anode was would beat, but the whole strip. The thing requiring explanation here is not the rhythm in the anodic region, but the anomaly of no rhythm about the cathode. It is clear that the constant current in this region inhibits the action of the sodium chloride solution, while permitting it to act normally in the anodic space. This explanation will apply to heart, intestine, and stripped muscle equally. We know too little of the composition and nature of living substance to analyze

[1] KÜHNE: Archiv für Physiologie, 1860, p. 542.

[2] HERMANN: Archiv für die gesammte Physiologie, 1886, xxxix, p. 600.

these facts. But the fact that cathodic conditions interfere with the action of a solution of sodium chloride would suggest that anodic conditions should favor it. And what we know of the action of a current supports this idea in some respects. For example, the anode is the seat of oxidations, and oxidation we know is favorable to the development of a cardiac rhythm, as is shown by the action of solutions of hydrogen peroxide. At the cathode reduction takes place. Again, a constant current passing through a heart strip produces electrolytic changes. There is a sorting of ions. The positively charged sodium, calcium, and potassium, and other positive ions diminish about the anode, and this will favor a rhythm, as an excess of calcium and potassium interferes with its development; while negatively charged ions including chlorine and OH accumulate there. About the cathode the positively charged sodium, calcium, and potassium accumulate, and the excess of calcium and potassium will help to inhibit the working of the sodium chloride solution there. While this is going on, the moistening with sodium chloride renews this salt in the anodic space. When I wrote my first paper on this subject, I believed that it was the sodium ion that caused rhythmic activity in heart tissues. But Dr. Mathews'[1] work showing that the negative charged ion was the active agent threw me into confusion. As I had no means of deciding this point, in my second paper I was careful to discuss only sodium chloride, hoping that sometime conditions might arise that would enable me to experimentally test these conflicting ideas. The discovery of this anodic rhythm in heart strips at first seemed to be the desired means; but on closer inspection the conclusion is not established. It may support the contention of Mathews,[2] Neilson and Brown,[3] and Benedict,[4] who are all agreed that the negatively charged ion is the active one. But I am convinced that the conditions of experimentation do not establish this. The first point is this. If the matter of a rhythm under the influence of a constant current was simply a question of kind and ion distribution, then the rhythm should appear in a heart strip traversed by a constant current without the addition of the sodium chloride solution. In this case we produce electrolytic effects, but no rhythm results. The fact that the sodium chloride must be added under all conditions

[1] A. P. Mathews: Science, 1903, xvii, pp. 729-733.
[2] Mathews: *Loc. cit.*
[3] Neilson and Brown: This journal, 1904, x, p. 225.
[4] Benedict: This journal, 1905, xiii, p. 192.

to get the rhythm indicates that it is specific for the power we call rhythmic as seen in heart and other tissues.

This electrolytic effect enables us to understand some points not easy to explain otherwise. A very small amount of sodium chloride will produce the effect under the influence of the constant current because of the favoring accessory conditions developed about the anode. It also enables us to understand the conditions observed by Pickering[1] on the embryonic heart. He noticed that with a current of a certain strength passing through the chick's heart reversal of current direction changed the direction of the rhythm, evidently because the cathode inhibited the rhythm at its end, while the anode permitted the normal rhythm to originate unhindered and spread from there over the rest of the heart. It also enables us to explain Langendorff's[2] observation that varying the strength of the constant current traversing a heart modifies the rate of the rhythm, probably because, as the current changes, the oxidations and other favoring conditions at the anode are increased for the natural rhythm, as they are for that originated under the influence of a solution of sodium chloride.

Before leaving this part of the subject I wish to prevent possible misunderstanding by distinctly stating that the views here advanced do not exclude the possibility of other solutions doing the same work as sodium chloride. It is not the only salt solution that can cause the production of a rhythm. Others can do the same. The action of this salt is discussed exclusively here because the experiments were made with it and also those described in the literature of the subject.

B. The action of the constant current on the apex of the frog's heart. — As so much work has been done on the apex preparation, in investigating the relation of the constant current to rhythm production, it seemed desirable to test our idea on this tissue also. The results are not so satisfactory as with turtle tissue because the tissue is feebly rhythmic. Under the conditions of our experiment it never develops a spontaneous rhythm, and we are compelled to stimulate it each time and judge of its rhythmic condition by the frequency of the beats accompanying the stimulus. If a stimulus produces a single beat, rhythmic power is poor, but from twenty-five to fifty or more beats with a single stimulus indicate considerable rhythmic power. Several sets of experiments were made. In the first our sodium

[1] PICKERING: Journal of physiology, 1896, xx, p. 221.

[2] LANGENDORFF: *Loc. cit.*

chloride idea was applied to the treatment of the apex as developed by Trendelenburg.[1]

Two preparations were used at the same time. Each was placed in a paraffin trough with non-polarizable electrodes at the ends. One trough was filled with a solution of lithium chloride, the other with one of sodium chloride. The wires for the constant current were so arranged that the current passed through one trough and then through the other. A graphite rheostat gradually varied the current strength from less than a milliampère up to ten or more milliampères. A key in the circuit permitted make and break stimulation at will. With this method it was found that the apex in the sodium chloride solution gave short rhythms when the current was strengthened and when it was made or broken. While the apex in the lithium solution would give but one rhythm during the experiment, after that, no matter how stimulated, the response was always a single beat. While making these rhythmic beats little puffs of blood were ejected into the lithium solution with each contraction, and after this blood was expelled the apex never again showed a rhythm in the lithium chloride solution. In other experiments the heart tips were not placed in a solution, but directly in contact with the non-polarizable electrodes in a moist chamber. The current of various strengths was sent through them in different directions, transversely and in both directions longitudinally. In some instances the apex was made to expel its blood by causing it to contract in filter paper before placing it on the electrodes. The tissues were not moistened, and so were influenced solely by the salt solutions contained in the electrodes and by the constant current traversing them. The electrodes used were moistened with Ringer's solution and with sodium chloride and lithium chloride solutions. And the number of times an apex gave more than a single beat where the current was strengthened or made or broken was taken as an index of its rhythmic power.

In one series of experiments with electrodes moistened with Ringer's solution the following results were obtained:

The strength of the current was changed	9 times
The current was made	86 "
The current was broken	86 "
The total number of chances for a rhythm was	181 "
An actual rhythm occurred	24 "
The tissue failed to respond to the M or B	33 "
And gave single contractions at M or B	124 "

[1] TRENDELENBURG: *Loc. cit.*

The highest number of beats in a rhythm was 69; the lowest, 2. These rhythms were most frequently seen at the make and when the current was suddenly strengthened.

A similar experiment with electrodes moistened with a sodium chloride solution gave results as follows:

Strength of the current was changed	9 times
The current was made	81 "
The current was broken	81 "
The total number of opportunities for a rhythm were	171 "
An actual rhythm occurred	7 "
The tissue failed to respond to a M or B, etc.	25 "
And gave single contractions at M & B, etc.	139 "

When the current strength was about one-fourth of a milliampère, the conditions seemed most favorable for rhythmic development. The highest number of beats counted in a rhythm was 15; the lowest, 2.

In the experiment with electrodes moistened with lithium chloride solution,

The current was increased	9 times
The current was made	81 "
The current was broken	81 "
Giving a total number of chances for rhythm of	171 "
A rhythm occurred	3 "
The tissue failed to respond to stimulation	30 "
And gave single contractions when stimulated	138 "

The highest number of beats in a rhythm was 5; the lowest, 2.

In the three preceding sets of experiments apices were used full of blood just as they came from the heart. If the last experiment with electrodes moistened with lithium chloride is repeated with apices that have been previously washed out with a sugar solution, or that have been made to expel their contained blood by contracting on filter paper, then a rhythm is never seen, even when the tissues give good contractions at the make and break of the current in the majority of cases.

In many instances when the electrodes were moistened with lithium chloride, the effort was made to reverse conditions, and by adding Ringer's or sodium chloride to get the tissue into condition to show a rhythm; but every such attempt was a failure.

Throughout these experiments the effort was made to keep the electric current a constant factor. The variations in the results indicate the influence of the moistening solution, and they indicate that the latter is far more important for the development of a rhythm than the constant current.

II. Induction shocks. — Probably the experiments which have given most support and prominence to the idea that induction shocks can produce a rhythm in heart strips were those by Gaskell.[1] He suspended a ventricular strip, and passed through it feeble tetanizing shocks, and at the same time stimulated it at ten-second intervals with single induction shocks. And under the influence of these agents he says the contractility and irritability of the tissues improved rapidly, and after a time spontaneous beats appeared, which lasted long after the currents were removed. Gaskell thinks these agents are not absolutely necessary, but they hasten the development of the rhythm. To use his exact words, "Under the guidance of the interrupted current and the single induction shocks the rhythmical power inherent in the muscular strip has been developed and made manifest, the muscle has been taught to beat." In this experiment three factors are present. First, the strip is suspended and moistened with a solution of sodium chloride. Second, the strip is made rhythmically active by the application of effective induction shocks every ten seconds. And third, it is traversed at the same time by feeble tetanizing shocks. Which of these is the essential factor in calling out the rhythm? Gaskell ignored the first factor entirely, and laid stress on the second and third conditions. Here, again, it is easy to prove that we are dealing with a sodium chloride developed rhythm, and also that the second and third factors of themselves are absolutely unable to develop a rhythm. Had the strip been simply suspended and moistened with the sodium chloride solution, the rhythm would have developed just the same. This view has been established by a series of experiments in which each factor was tested separately.

A. Slowly repeated induction shocks. — Bowditch[2] seems to have been the first to show that slowly repeated induction shocks of a certain strength have power to start independent beats in the heart tissue. And since his paper appeared a large amount of work has been done on this subject, but neither Bowditch nor any other experimenter has made the experiments so as to exclude the rôle of sodium chloride.

[1] GASKELL: Journal of physiology, 1883, iv, p. 54.

[2] BOWDITCH: Berichte der koniglichen sächsischen Akademie, 1871, p. 662.

And the evidence to-day is not of such a kind that we can assert positively that slowly repeated induction shocks can start a rhythm. And when tested experimentally the idea is plainly without support, as was demonstrated in the following manner. Two heart strips prepared and mounted in the usual way were made a part of the secondary circuit of an induction coil. In the primary of this coil was inserted an interrupting clock which gave shocks at the rate of one per minute. The strength of current used was varied, but was ordinarily that giving a good contraction of the tissue. When such an experiment is started, both strips are stimulated and give a contraction for each shock; and the strip in the moist chamber never shows any other activity. The other strip in air and moistened with the sodium chloride solution presently begins to show interpolated spontaneous beats, which rapidly increase in number so that the strip is soon in full rhythmic activity, with the beats due to the individual shocks entirely obscured (Figs. 1 and 2). Any one observing such an experiment and believing that the bathing solution was neutral would certainly be impressed with the rhythm-producing powers of this method of stimulation. And yet the stimulation without the bathing solution has no such powers, and the solution alone has. It is clear that this rhythm is another instance of a sodium chloride rhythm, and the old idea is an example of misplaced credit. I have said we never get a rhythm with this kind of stimulation; and it is true; but it is also true that sometimes the strip seems to make a tremendous effort to be rhythmical without success. In one experiment in a series of eight a strip showed a few feeble extra beats in between those produced by the stimulation. It seemed as though this trip acted like an apex preparation and responded to each shock by more than single contraction.

B. Tetanizing shocks as rhythm producers. — Schoenlein[1] has shown that tetanizing currents cause a rhythmical activity in stripped muscle, but Gaskell[2] is chiefly responsible for the belief that a heart strip can be made to beat rhythmically, as has been stated The experiments he describes, like the others, were made with strips moistened with sodium chloride, and when this factor is eliminated the result is not the same. This was determined by a series of experiments like those with single shocks. In every experiment it was found that the tetanized strips showed a rhythm when moistened with

[1] SCHOENLEIN: Archiv für Physiologie, 1882, p. 369.

[2] GASKELL: *Loc. cit.*, pp. 106 and 108.

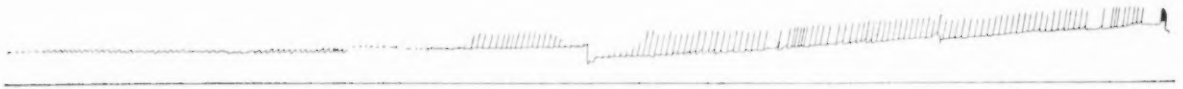

FIGURE 1. — From a strip of the ventricle suspended in a moist chamber and stimulated at regular intervals by single induction shocks.

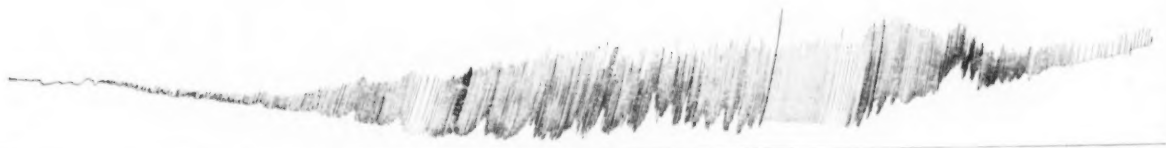

FIGURE 2. — From a strip of the ventricle stimulated by single induction shocks and moistened with a solution of sodium chloride. The tracings in Figs. 1 and 2 cover about four hours.

a solution of sodium chloride. If this was omitted, as in the case of the strip in the moist chamber, the rhythm failed to appear. Hence this rhythm must be attributed to the salt solution and not to the electric stimulation. The heart strips in these experiments were subjected to the tetanizing currents twenty-four or more hours, and some of them developed certain peculiarities that are worth mentioning. In a series of twelve experiments five tetanized strips in the moist chamber not treated with sodium chloride solution showed a marked loss of tone, and no other change was visible for the twenty-four or more hours that the experiment lasted. But seven of the twelve under the same conditions developed rhythmical variations in

FIGURE 3. — The tone changes seen in a heart strip stimulated by tetanizing shocks.

tone of a striking character. These variations are dependent on the electrical stimulation, for they stopped when the tetanizing shocks were discontinued. Such tone variations are usually irregular, but at intervals for a short time they may be so regular in a tracing as to simulate a real cardiac rhythm, so much so that at first I was inclined to believe they constituted a very slow rhythm. They vary in strength from extremely small undulations up to those that are one-third or one-fourth as strong as the contractions seen in a true rhythm (Fig. 3). There is good evidence for believing that this phenomenon is distinct from what we ordinarily call the cardiac rhythm. For one thing, its rate is different in all cases. In those instances where these changes occurred most rapidly they were seen at the rate of one undulation in one minute. Ordinarily they were slower, say one in twenty minutes. I have never seen the cardiac tissue of the turtle under the same conditions beat with a rhythm as slow as this. But aside from this, there is good proof of another kind that these changes are not a true rhythm. When a strip showing these tone changes is treated with a sodium chloride solution, the tone changes persist and a true rhythm develops on top of them, and for a time both can be seen; the true rhythm with its rapid beats is superimposed on the slower tone changes without affecting the latter. It is surprising how easily the true rhythm can be added to the tone changes. In

one experiment a strip had been showing these tone changes for twenty-eight hours, when five drops of sodium chloride solution were permitted to fall over the strip and five minutes later the tissue showed a good regular rhythm. Experiments like this show in the most striking manner the importance of sodium chloride in these rhythms. And if possible, it is more striking in the case of strips that are absolutely rhythmless for forty-eight hours, and then almost immediately begin beating when the sodium chloride is added.

Blood pressure tracings frequently show undulations that have about the same relation to the heart beats as these tone changes have to the rhythm in tetanized heart strips. In the blood pressure tracing the undulatory variations in pressure have been explained in various ways. These experiments would seem to indicate that the heart tissue under certain conditions works in a way to produce such an effect, and this precludes the explanation that refers their origin to peripheral circulatory changes.

Mechanical Agents as Rhythm Producers.

There is an extensive literature supporting the idea that internal pressure or tension of the heart walls will modify its rhythm and produce rhythmic action in the non-beating apex. Ludwig and Luchsinger[1] were among the first to call attention to this, while Gaskell[2] has applied the idea to the heart strips. He states that a heart strip merely suspended in a moist chamber without the application of any foreign liquid will after a time begin to beat rhythmically. Professor Gaskell has personally assured me that when he used this term "foreign liquid" he did not include sodium chloride. He always used this to prevent his preparations from drying. We have in this statement the evident source of the rhythm seen under the conditions described. I have repeatedly suspended heart strips in a moist chamber, and have never seen one show a rhythm without being moistened with sodium chloride, and I have hardly ever seen such a strip fail to beat rhythmically when supplied with this solution. The same is true, no matter how the tension is varied by changing the weights from exceeding light to heavy. We have here instances

[1] Ludwig and Luchsinger: Archiv für die gesammte Physiologie, 1881, xxv, p. 232.

[2] Gaskell: Journal of physiology, 1883, iv, p. 54.

of the failure of uniform tension to produce a rhythm, and we must again refer the rhythm that appears under such conditions to the sodium chloride solution used along with the tension. Nor is intermittent tension any more efficient. Sometimes when a heart strip is immersed in a solution of sodium chloride it will not beat for a long time. Such a strip, if alive, can nearly always be made to beat by simply giving the far end of the lever a light sharp tap. This quickly stretches the strip and arouses it to rhythmic activity. Such a procedure can be repeated for hours with a strip deprived of sodium chloride without causing a rhythm to appear, but it very quickly appears in a strip simply suspended in air and similarly treated, provided it is kept moist with a solution of sodium chloride. It has been claimed that suspension tension in a heart strip is not comparable to hydrostatic pressure in the heart cavities, but judging from the analogy of stripped muscle, such tension should put the strip in good physiological condition, and so help it to develop its rhythm, if this were possible, without the sodium chloride solution.

So far as I am aware, all experiments on the heart or the apex which have demonstrated that tension could produce rhythmic activity have been made with structures filled with sodium chloride solution, blood serum, or a salt solution having equivalent powers. There is no reason, then, to attribute results to the mechanical agent in such cases.

Chemical Agents.

In this paper no attempt will be made to discuss the various chemical agents that are said to produce rhythmic activity. They are so numerous and important as to warrant a special paper. We may justly call attention to this point. The preceding experiments would justify skepticism regarding the powers of any chemical, when that chemical's claims to insertion in the class of rhythm producers rest on experiments in which a solution of sodium chloride has been used or blood serum. And if the literature of the subject is reviewed, it is surprising how few the experiments are with chemicals that are not open to this objection. It would seem probable that those agents which, when used with a solution of sodium chloride, develop a rhythm are not necessary rhythm producers, but simply substances that do not interfere with the action of the sodium chloride.

Conclusion.

It would seem that certain agents, such as the constant current, induction shocks, and mechanical tension, which have been considered capable of producing activity in *heart strips*, owe their power to the solution of sodium chloride used with them.

ON THE ACTION OF ADRENALIN ON THE CEREBRAL VESSELS.

By CARL J. WIGGERS.

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ALTHOUGH the constricting effect of adrenalin upon the vessels of most organs of the body has been conclusively demonstrated, its exact action on the cerebral vessels, owing largely to the difficulties attendant upon a study of the cerebral circulation, is still a matter of dispute.

Bayliss and Hill[1] could discover no constricting effect of adrenalin as manifested either in a change of intracranial or cerebral venous pressure; nor could Hill and Macleod,[2] later, either by direct inspection of the pial vessels or by measuring the pressure in the Circle of Willis, convince themselves that adrenalin had any active effect on the cerebral vessels. Gerhardt,[3] observing that an injection of adrenalin caused, at the same time, a rise of pressure in a jugular vein, a fall in a renal vein, and a dilation of the retinal vessels, concluded that nothing more than a passive dilation occurred within the cranium. The experiments of Spina[4] gave a similar impression, for he noted an increased hyperemia and bulging of the brain from the trephine hole, together with an increased outflow rate from a cerebral vein. In addition, however, he noted that these phenomena became more marked if the cervical cord was cut previous to the injection, the degree depending on the height at which the cord was cut. He ex-

[1] BAYLISS and HILL: Journal of physiology, 1895, xviii, p. 334.

[2] HILL and MACLEOD: Journal of physiology, 1901, xxvi, p. 394.

[3] GERHARDT: Archiv für experimentelle Pathologie und Pharmakologie, 1900, xliv, p. 161.

[4] SPINA: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 204; Wiener klinische Wochenschrift, 1897, x, p. 1047.

plained this by assuming the removal of a normally acting vasomotor influence.

Opposed to these experimenters are those who obtained, what seemed to them at least, results indicating an active constriction of the cerebral vessels by adrenalin. Thus Pick [1] found that an injection of adrenalin caused a diminution in outflow from the external jugular vein; and Cyon [2] noted that it caused a blanching of the dura. Biedl and Reiner [3] recorded a temporary decrease in drop-rate from a cerebral vein and an increased pressure in the Circle of Willis, after a direct injection of adrenalin. Last year Kahn [4] reported that adrenalin, injected into the cerebral end of the internal carotid caused a temporary constriction of the retinal vessels, followed by a dilation; v. Neujean [5] found that the combined outflow from the external and internal jugulars was diminished by the drug; and Brodie and Dixon hint at positive results in their paper.[6] This year Wiechowski [7] from pressure measurements in the cerebral end of the internal carotid, also, ascribes to adrenalin the power of constricting the cerebral vessels.

Methods of Studying the Cerebral Circulation.

Perhaps no other organ of the body is less adapted to an experimental study of its circulation than the brain, and consequently a large number of ingenious methods have been devised in attempts to overcome the attending difficulties. Each one of these methods, however, regardless of the technical accuracy displayed in their employment, can be placed into one or more of several groups according to the experimental principle involved. These principles, seven in number, are: —

[1] Pick: Archiv für experimentelle Pathologie und Pharmakologie, 1899, xlii, p. 399.

[2] Von Cyon: quoted by v. Neujean.

[3] Biedl and Reiner: Archiv für die gesammte Physiologie, 1900, lxxix, p. 158.

[4] Kahn: Centralblatt für Physiologie, 1904, xviii, p. 153.

[5] v. Neujean: Archives internationales de pharmacodynamie et thérapie, 1904, xiii, p. 67.

[6] Brodie and Dixon: Journal of physiology, 1904, xxx, p. 476

[7] Wiechowski: Archiv für experimentelle Pathologie, 1905, lii, 5, p. 389; Centralblatt für Physiologie, 1905, xix, p. 178.

1. Direct inspection of the pial vessels; employed by Schiff,[1] Callenfels,[2] Ackermann,[3] Nothnagel,[4] Riegel and Jolly,[5] Cramér,[6] Dogiel,[7] Krauspe,[8] Hill and Macleod,[9] and Spina.[10]
2. Recording the intracranial, or cerebro-spinal pressure changes; employed by Falkenheim and Naunyn,[11] v. Schultén,[12] Salathé,[13] Dean,[14] Biedl and Reiner,[15] and Hill.[16]
3. Recording the changes in diameter of the brain, employed by Roy and Sherrington.[17]
4. Recording the changes in cerebral venous pressure; employed by Hill [16] and Gerhardt.[18]
5. Determining the changes in rate of venous outflow from the brain; employed by Biedl and Reiner,[15] Gaertner and Wagner,[19] Spina,[10] Pick,[20] v. Neujean.[21]

[1] SCHIFF: Untersuchungen zur Physiologie der Nerven System.

[2] CALLENFELS: Henle und Pfeiffer's Zeitschrift, vii, quoted by Nothnagel.

[3] ACKERMANN: Archiv für pathologische Anatomie und Physiologie, 1858, xv, p. 401.

[4] NOTHNAGEL: Archiv für pathologische Anatomie und Physiologie, 1867, xl, p. 203.

[5] RIEGEL and JOLLY: Archiv für pathologische Anatomie und Physiologie, 1871, lii, p. 218.

[6] CRAMER: quoted by Spina, Inaugural Dissertation, Dorpat, 1873.

[7] DOGIEL: quoted by Hill, The Physiology and Pathology of the Cerebral Circulation, 1896.

[8] KRAUSPE: Archiv für Physiologie, 1874, lix, p. 472.

[9] HILL and MACLEOD: Journal of physiology, 1901, xxvi, p. 394.

[10] SPINA: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 204; Wiener klinische Wochenschrift, 1897, x, p. 1047.

[11] FALKENHEIM and NAUNYN: Archiv für experimentelle Pathologie und Pharmakologie, 1887, xxii, p. 261.

[12] VON SCHULTÉN: Archiv für Ophthalmologie, 1884, xxx, 3, p. 1; Archiv für klinische Chirurgie, 1885, xxxii, p. 455.

[13] SALATHÉ: Traveaux du laboratoire de Marey, 1876.

[14] DEAN: Journal of pathology, London, 1892, i, p. 26.

[15] BIEDL and REINER: Archiv für die gesammte Physiologie, 1900, lxxix, p. 158.

[16] HILL: The Physiology and Pathology of the Cerebral Circulation, 1896.

[17] ROY and SHERRINGTON: Journal of physiology, 1890, xi, p. 85.

[18] GERHARDT: Archiv für experimentelle Pathologie und Pharmakologie, 1900, xliv, p. 161.

[19] GAERTNER and WAGNER: Wiener medicinische Wochenschrift, 1887, pp. 602, 639.

[20] PICK: Archiv für experimentelle Pathologie und Pharmakologie, 1899, xlii, p. 399.

[21] V. NEUJEAN: Archives internationales de pharmacodynamie et thérapie, 1904, xiii, p. 67.

6. Measuring the pressure changes in the Circle of Willis; employed by v. Schultén,[1] Arloing,[2] Hürthle,[3] Cavazzani,[4] Biedl and Reiner,[5] and François-Franck.[6]
7. Measuring the changes in rate of outflow from the Circle of Willis; employed by Jensen.[7]

With such masterful discussions and criticisms of these principles and the methods of employing them as are given us in the writings of Hill[8] and Biedl and Reiner,[5] anything more than a brief discussion of a few points appertaining directly to this research is unnecessary.

The results of these experimenters, whether obtained by directly observing the color or calibre changes in the pial vessels, or by recording changes in the diameter or volume of the brain, changes in the cerebro-spinal, cerebral arterial, or cerebral venous pressures, or, lastly, changes in the rate of arterial or venous outflow, are all based on changes in the flow of blood in the cerebral vessels. As this, however, is not alone governed by local constriction or dilation, but markedly modified by changes in general arterial and venous pressures, it follows *that the stimulation of any nerve acting on the general vascular system or the injection of any drug with a similar action may passively so modify the cerebral circulation as to obscure any active reaction that might occur.* To determine whether the cerebral change was active or merely of such a passive nature, certain workers have recorded changes in general arterial and venous pressures synchronous with the cerebral change. While much information is given by this procedure when applied to organs in which a marked reaction occurs, its differentiating value is decidedly limited when active vasomotion is but weak, as in the brain. Here a cerebral change opposite in direction to what the general pressure change would induce, can hardly be expected, and hence changes alike in direction cannot be interpreted as proving the absence of active cerebral vasomotion.

[1] Von Schultén: Archiv für Ophthalmologie, 1884, xxx, 4, p. 61.

[2] Arloing: Archives de physiologie, 1889, p. 115.

[3] Hürthle: Archiv für die gesammte Physiologie, 1889, xliv, p. 578.

[4] Cavazzani: Archives italiennes de biologie, 1891, xvi, p. 23; Centralblatt für Physiologie, 1895, viii, p. 25.

[5] Biedl and Reiner: Archiv für die gesammte Physiologie, 1900, lxxix, p. 158.

[6] François-Franck: Journal de physiologie et de pathologie générale, 1899, i, p. 1206.

[7] Jensen: Archiv für die gesammte Physiologie, 1904, ciii, p. 196.

[8] Hill: The Physiology and Pathology of the Cerebral Circulation, 1896.

To become more specific, an increased hyperemia, an increased volume of the brain, an increased intracranial or cerebral venous pressure, an increased rate of flow from a sinus or an increased pressure in the Circle of Willis, occurring with a rise of general arterial and a fall of general venous pressure, either signify that the cerebral vessels have no power to respond actively to stimulants and that they passively follow the general pressure changes, *or* that the cerebral vessels do actively contract, but too weakly to counteract the passive influence of general pressure change. As long as we possess no unit by which degree of change in the general and cerebral circulations can be compared, we possess no means of determining which of these conditions obtains. As a corollary to these statements, it follows that the only way to obtain results free from passive influences is to remove the factors producing them; and then the method of measuring the cerebral change is of small import provided only that it is practical to apply.

When the rate of outflow from the cerebral veins is used as a criterion of cerebral vasomotion, it is essential that the entire outflow be measured, unmixed with fluid from other regions. The conclusions of those experimenters who felt justified in estimating the outflow from the cranial cavity by measuring the flow in some such veins as the facial or external and internal jugulars, are of course entirely without value, as these veins drain so many extracranial areas. Nor is the measurement of a portion of the cerebral outflow, even if direct from a sinus, reliable, as the rate of flow from such an opening must vary with any change of resistance in the natural outflow channels. A synchronous record of the general venous pressure, as Hill suggests, would only aid if this were found not to vary; otherwise, as before pointed out, it would possess no differentiating value. But, even were one willing to agree with the assertion that the influence of this resistance change can practically be neglected, provided the resistance at the artificial opening be small, there would still remain other factors which in themselves are sufficient to rob the method of any value it might otherwise possess — the abnormal conditions produced by continually lessening the volume of blood in the animal and the variable and uncontrollable general pressures that must have accompanied such experiments.

Method Employed in this Research.

In this research the pressure in the arteries supplying the brain as well as the resistance to venous outflow was maintained constant

by perfusing the isolated brain with an artificial circulation fluid, and the entire outflow from the brain was accurately recorded.

The artificial circulation apparatus. — The apparatus by means of which the perfusion was accomplished is shown in Fig. 1. It consists of three communicating bottles which I have termed, respec-

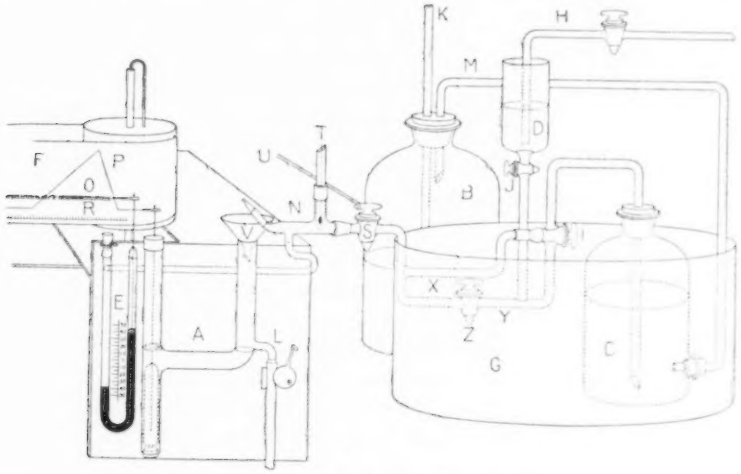


FIGURE 1. — *Diagram of apparatus.* *A*, the outflow recording apparatus into which fluid drips by funnel *V* and is let out by side tube *L*. *B*, an oxygen bottle communicating by tube *K* with a pressure bottle (not shown), and by tube *M* with the solution bottle *C*. *D*, the small reservoir from which tube *V* was filled while fluid continued to flow through tube *X*. *I*, *Z*, *J*, and *S*, stopcocks referred to in description. *U*, a rod connected with a cam actuated by a motor. *H*, a tube connected with a second oxygen container (not shown) from which fluid in *D* was saturated with oxygen. *E*, a mercury manometer measuring the lateral pressure in tube *N* leading to the head, fixed above the funnel *V* by a clamp. *F*, a loop of smoked paper revolving about 2 drums. On this were written curves *O* by the mercury manometer, *P* by the outflow recording apparatus, and *R* by the time signal. *G*, water bath. *T*, a thermometer.

tively, the pressure, gas, and solution bottles. The pressure bottle (not shown in diagram) was so arranged that air could enter, to displace the outflowing water, only through a tube projecting nearly to the bottom, thus, on the principle of the Marriotte flask, maintaining a constant pressure level. The height of this bottle was varied by means of a rope and pulley. Water flowed from an opening near the bottom of this bottle into the gas bottle (*B*), and displaced the oxygen that it contained to the bottom of the solution bottle (*C*). Here it

saturated the Locke's solution by bubbling through it and then created a pressure to drive it on.

As already intimated, the perfusion fluid generally used was Locke's solution, made according to the formula given in the Centralblatt für Physiologie, 1900, xiv, p. 670. In some few experiments, however, blood drawn from the animal, defibrinated and diluted two times with Ringer's solution was substituted, and the oxygen in these cases supplanted by air. The temperature of the solution, as indicated by a thermometer (*T*), was maintained at 40° C. by placing the solution bottle and outflow tubes (*X* and *Y*) in a water bath. Through these latter tubes fluid reached the stopcock (*S*), which was alternately opened and closed by a rod and cam driven by a motor. This supplied the brain with a pulsating current which at least minimized the edema always associated with perfusion experiments. A small amount of glass wool introduced just before the moving stopcock (*S*) kept back small bubbles such as appear on the sides of tubes when fluid in them is heated. This apparatus supplied, at a constant temperature and pressure, a pulsating stream of fluid containing such salts, nutrient material, and oxygen, as are required to sustain the life of cells for a considerable time. This was tested by control experiments on the heart and kidney.

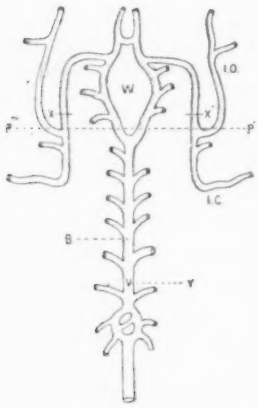


FIGURE 2. — Scheme of the cerebral circulation, drawn from injected arteries. *B*, the basilar; *W*, the Circle of Willis; *I. C.*, the internal carotid; *I. O.*, the internal ophthalmic; *P–P'*, level of anterior border of pons; *X* and *X'*, points of ligation of the internal carotids. *Y*, the point where the cannula was inserted.

Method of establishing the cerebral circulation. — A large number of preliminary experiments convinced me that it is impossible to limit the circulation to the brain by means of an extracranial insertion of the cannula. The paths of collateral circulation are too many and too inaccessible to be excluded by ligation. This becomes evident on considering the anatomical relations of the cerebral vessels.

The basilar artery in the dog (Fig. 2) arises in the lower medullary region from a circular, more or less anastomosing arrangement of blood vessels, supplied by the vertebrals and the cerebro-spinal

arteries, sub-branches of the occipitals. After coursing straight forward and supplying the medulla and cerebellum, the basilar artery divides at the anterior border of the pons and forms the Circle of Willis with the internal carotid. This artery, after it has passed through the bony carotid canal, passes forward in the carotid sulcus within the cranial cavity, and gives off several branches before piercing the dura. First, it gives off one or two small branches anastomosing with the middle meningeal, and then, at about the same level that the basilar artery divides, a somewhat larger internal ophthalmic branch. The chief ophthalmic artery in the dog is derived from the internal maxillary.

Thus the inaccessible anastomoses of the internal carotid with the vessels of the orbit and middle meningeal, and of the vertebrals with the cervical vessels, render the sole perfusion of the brain impossible unless these communications are ligated intracranially. This was done in this research. A large dog, morphinized by the injection of 5 grains of morphine sulphate and for just a moment completely anesthetized with ether was quickly decapitated. The head was then roughly and speedily skinned, and the trachea, tongue, and superfluous muscles rapidly cut away. The base of the skull was then sawed and broken open as far forward as the anterior border of the pons. Some seven minutes were required for this portion of the work, but the second part of the operation progressed more slowly. This consisted in chipping away the bones on both sides of the pons so as to expose to view the internal carotid with its branches. Just anterior to the origin of the internal ophthalmic branch, both internal carotids were ligated (Fig. 2, *X*), and a cannula then inserted into the basilar artery at *Y*. The entire operation required from fifteen to thirty minutes; and as far as could be learned from injection there remained no possible channel by which an extraneous communication with the cerebral vessels could occur. Thus all the fluid entering the basilar artery through the cannula had to pass through the vessels of the brain.

Method of obtaining and recording the venous outflow. — With the venous sinuses at the base of the skull destroyed by the operation, the total venous outflow was obtained by the following method: When ready for perfusion, the head was clamped in the nose-up position above a perfectly clean funnel connected with an apparatus which measured the outflow from the cerebral veins. The fluid, carried by gravity, dripped from the most dependent portion of the

preparation into the funnel. In this manner all the fluid passing through the brain, whatever the path taken after it left the smaller veins, was finally collected, measured, and graphically recorded.

The apparatus by means of which the recording was accomplished is labelled (*A*) in Fig. 1. It may be described as a U tube with its left limb continued downward. Fluid collecting into the right limb of the tube from the funnel, maintained a constant level in both limbs of the tube as long as the side tube (*L*) was open. When this was closed by a shut-off, the fluid rose in both limbs of the U, and in so doing carried with it, in the left limb, a cork float provided with a thin glass rod carrying a writing point, which traced a vertical line upon a smoked surface. The float was kept from sucking against the sides of the tube by means of two guides. The upper one, capping the top of the left limb, guided the glass rod bearing the writing point; while the other, placed below the float, guided a downward continuation of this rod. Callibration showed that each c.c. of fluid leaving the preparation caused a vertical record of 5 m.m. on the drum. Thus 20 c.c. of fluid could be recorded in one continuous record, and then a new one be rapidly started after depleting the instrument through the side tube.

On each curve there were also recorded, as Fig. 1 and the curve of Fig. 3 show, by an electric signal the time in seconds, and by a mercury manometer the oscillating lateral pressure in the tube supplying the basilar artery. The pointers were so arranged that the line written by the time signal supplied the zero line for the pressure curve.

Method of introducing the adrenalin. — It was essential that the adrenalin be so introduced as not to alter the general character of the circulating fluid, its temperature or pressure. Fig. 1 shows how this was accomplished. Fluid from the solution bottle could arrive at the moving stopcock (*S*) either through tube *X* or *Y*, depending on which way the double stopcock (*I*) was turned. While fluid continued to flow through tube *X* the adrenalin was introduced into tube *Y* in the following manner: The adrenalin (P. D. & Co.'s solid preparation) was dissolved in Locke's solution by heating, and then placed in the small reservoir (*D*), and saturated with oxygen through tube *H*, which connected with a second oxygen bottle. By then opening stopcock *J* and so turning the double stopcock *Z* as to be in external communication, tube *Y* filled itself. The stopcocks were then closed. The solution, introduced quite warm, was allowed to acquire the temperature of the water-bath, and then stopcock *I* rapidly turned from path *X* to path *Y* while the moving stopcock *S* was closed.

Results and their Discussion.

Ten successful experiments were so performed that subsequent injection proved that the cerebral vessels had no extracranial communications. In every case the constricting effect of adrenalin was sufficiently marked to be evident at the time of the experiment. After a variable latent period (partly due to the fact that 10 c.c. of fluid had to be displaced before the adrenalin reached the cerebral vessels) the

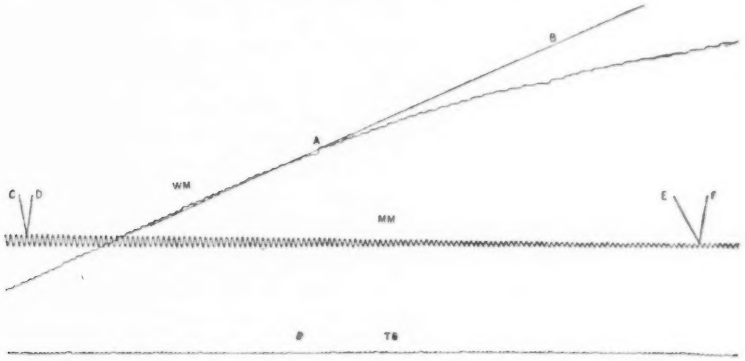


FIGURE 3. — Portion of a record taken on March 4, 1905, showing the effect of adrenalin. *MM*, the record of oscillations from mercury manometer; *WM*, line written by the drip recording apparatus; *TS*, time in seconds; *A-B* drawn to show the change in angle assumed by outflow curve. Lines *C*, *D*, *E*, and *F* drawn by prolonging the ascending and descending limbs of two separated oscillations. While lines *D* and *F* are practically parallel, the differences in slant of *C* and *E* are apparent.

outflow curve presented certain characteristic changes. Fig. 3, which is a portion of such a record, illustrates their general character. The line traced by the drip-registering apparatus assumed a more acute angle, and the small oscillations of this line, representing the individual drops coming from the preparation, followed each other much less rapidly, indicating a diminution in outflow rate. As the tracing of the mercury manometer showed no change of pressure to account for this diminution, it could only signify an active constriction on the part of the cerebral vessels.

Additional proof of constriction was found in that the oscillations of the mercury manometer changed in size and form. Fig. 3 illustrates how these oscillations markedly decreased in size, and also how much more gradual the descending limb of an oscillation became. The

reasons for assuming that this represented evidence of cerebral constriction are clear. When fluid is supplied to the brain under a constant central pressure regularly interrupted by a stopcock, as in this apparatus, the mercury column of a lateral pressure manometer will rise whenever the stopcock is opened and fall during the period it remains closed, for the tubes are then drained of their fluid by the cerebral vessels. The extent and rate of the fall depend on the amount of fluid allowed to flow through the cerebral vessels during the diastolic period, and this in turn is governed solely by the degree of constriction present.

To sum up, each curve contained four proofs that a constriction followed the injection of adrenalin, each one checking the other. These proofs were, on the venous side, the diminished outflow volume and the slower succession of individual drops; and on the arterial side, the diminution in size of the pressure oscillations and the more gradual fall in pressure during the diastolic period.

After having been fixed and dried, the curves were mathematically analyzed. At equal intervals of time (usually thirty seconds) ordinates were drawn intersecting the outflow curve and, from each such point of intersection, lines perpendicular to these were extended as far as the ordinate following. In this manner the actual vertical height to which the float had risen during such time intervals could be expressed in millimetres and compared with other periods by plotting these values in curve form. Curves II and III of Fig. 4 show such curves. Curve II evidently shows that the injection of adrenalin at a period when the outflow rate was practically constant, caused, after a latent period of ninety seconds, a diminution in outflow rate from 5 c.c. (25 mm.) to 1 c.c. (6 mm.) per thirty seconds, and that by subsequent perfusion with Locke's solution this again increased to 3 c.c. (16 mm.).

These changes were closely followed by those of Curve I, which represent the variations in height of the arterial oscillations as they occurred from time to time.

The fact was constantly observed in such plotted curves that the outflow never regained its former rate. Three possible causes for such a diminution must be considered: first, the pressure of an edema on the smaller vessels; secondly, the constricting effect of a gradual cooling of the head; and, thirdly, an after-action of the drug.

Several facts are opposed to the assumption that the first of these factors was the cause of the diminished outflow. In the first place,

the edema in these experiments, owing to the use of a pulsating stream, was not very marked, and then edema, while it certainly produces a slowing in outflow rate, never does so as rapidly as these curves show. This was demonstrated by two control experiments.

The method has been severely criticised in this laboratory, in that no provision was made to keep the head warm, — an essential of all perfusion experiments. It was pointed out that the gradual cooling of the head might be the means of inducing a progressive constriction. The writer, however, always maintained that no such cooling occurred, because at the close of several experiments a thermometer pushed directly into the brain substance registered a temperature only two or three degrees below that of the perfusion fluid. In other words, the warm chamber was supplied by the skull, which conducted heat but poorly. Yielding, however, to the criticism that evaporation from the small exposed portion resulted in a cooling, this was protected in two experiments by a small coil through which water at 50° C. flowed. As this yielded no different results, but only served to make the technic more awkward, it was abandoned for the former method of protection, — by covering this portion with a moist pad of cotton. At present I see no facts in support of the assumption that cooling was the cause of the diminished outflow.

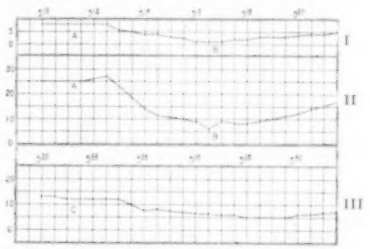


FIGURE 4. — Plotted curves from an experiment made on Feb. 8, 1905. Curve I represents changes in size of arterial pressure oscillations. Curve II shows synchronous changes in venous outflow rate. Curve III similar to Curve II represents a plot of data from the latter part of the same experiment. The abscissae give the time, and the ordinates give the distance that the float moved. 5 mm. equals 1 c.c. At *A* adrenalin was turned on; at *B* the maximal effect was produced, and at *C* a second injection was made.

It only remains, then, to assume that adrenalin possessed an after-action. In harmony with such an assumption is the fact observed in several experiments and plotted in Curve III of Fig. 4, that a second injection of adrenalin produced a decrease to exactly the same point as the first, irrespective of the rate before the injection.

It becomes interesting, at this point, to compare these results with those obtained by S. J. and C. Meltzer[1] in their researches

[1] S. J. and C. MELTZER: This journal, 1903, ix, p. 147.

on adrenalin. These workers found a marked difference in the reaction of the ear vessels to adrenalin, when the vasomotor nerves to these vessels were intact and cut. These differences may be briefly reviewed.

When Vasomotor Nerves were Cut.	When Vasomotor Nerves were Intact.
Latent period before constriction occurred ¼ to 2 minutes.	Latent period before constriction occurred 10 to 15 seconds.
Maximum constriction slowly reached.	Maximum constriction occurred in a few seconds after onset.
Constriction lasted for hours, sometimes till next day.	Constriction lasted 4 to 7 minutes.
Never was there a dilation of vessels beyond normal.	Constriction was followed by a dilation lasting several minutes.

It is not necessary to more than indicate the general similarity in the results of these two researches. The reactions to adrenalin which these experimenters found characteristic of the ear vessels when deprived of their central nervous influence, are also manifested by the cerebral vessels, as the curves of Fig. 4 show, — the long latent period (thirty seconds when corrected for period of apparatus), the slow production of maximal constriction (four minutes, Curve II; six minutes, Curve III), and the long after-action.

We can thus safely draw the conclusion that adrenalin acts upon the cerebral vessels to cause constriction in the same manner as on the vessels of other organs, and were one unquestioningly to adopt the view, so ably put forward by Brodie and Dixon,[1] that adrenalin acts on the nerve terminations and not on the muscular elements of the vessel walls, one might see in these results physiological evidence of cerebral vasomotor nerves. Elliott,[2] in a recent article on the action of adrenalin, makes the definite statement in his conclusions: "A positive reaction to adrenalin is a trustworthy proof of the existence and nature of the sympathetic nerves of an organ." Certainly the reaction of the brain vessels to adrenalin is favorable to the view of the existence of cerebral vasoconstrictor nerves, and it is hoped that positive evidence on this important question may be soon obtained.

[1] Brodie and Dixon: Journal of physiology, 1904, xxx, p. 476.
[2] Elliott, T. R.: Journal of physiology, 1905, xxxii, p. 466.

Conclusions.

1. By perfusing the isolated brain under constant temperature and pressure with a suitable solution, and continuously recording the entire venous outflow, the effect of influences which alter the size of the brain vessels can be definitely ascertained.

2. The application of an adrenalin solution to such a preparation and in such a way as not to introduce factors themselves conducive to outflow change, caused reactions which corresponded to those obtained from the vessels of other regions.

3. These reactions, if induced through the stimulation of vasomotor nerve ends, as claimed by certain workers, supply physiological evidence of the existence of such nerves.

This work has been carried on during the last two years under the direction of Dr. W. P. Lombard, whose many valuable suggestions have contributed largely to the success of the work. I take this opportunity to express my appreciation.

A NOTE ON THE ELECTRIC CONDUCTIVITY OF BLOOD DURING COAGULATION.

By ROBERT T. FRANK.

[*From the Pathological Laboratory of the College of Physicians and Surgeons, Columbia University.*]

THE methods now in use for determining the coagulation time of the blood leave much to be desired. The most satisfactory apparatus is that of Brodie and Russell,[1] which takes advantage of the fact that the red blood cells of a drop of blood, when agitated by an air current, revolve uniformly until fibrin threads begin to form.

The present study was undertaken in the hope of elaborating a new method, which it was thought might serve to measure accurately the combination of physical and chemical changes occurring during clotting. This expectation has not been fulfilled, but the facts determined during the course of the work, though negative, were considered of sufficient interest to be recorded.[2]

The theoretical considerations were based upon certain properties possessed by substances in the colloidal form, especially in their relation to electrical conductivity. Fibrinogen, as found in the fluid blood, is a colloid, forming part of the other colloidal substances in the blood (albumens, globulins). Sjöqvist,[3] and Bugarszky and Tangl[4] have shown that albumens, when added to dissociable electrolytic solutions, reduce their conductivity. Blood is such a mixture,

[1] Brodie and Russell: Journal of physiology, 1897, xxi, p. 403.

[2] The experiments were performed during the winter of 1904. In the March number of this journal, 1905, appears an article by T. M. Wilson, Measurement of electrical conductivity for clinical purposes, in which the author has tried a similar experiment, though only a single determination was made. The result is identical with mine. I desire to thank Prof. John G. Curtis, of the department of physiology, and Profs. Prudden and Wood, of the pathological department, for many courtesies extended to me.

[3] Sjöqvist: Skandinavisches Archiv für Physiologie, 1895, v, p. 277.

[4] Bugarszky and Tangl: Archiv für die gesammte Physiologie, 1898, lxxii, p. 531.

for it contains numerous electrolytes (carbonates, chlorides, sulphates of sodium, potassium, calcium, etc.) in solution, and the above-mentioned colloids. Besides the dissolved constituents the blood contains solid particles (the red and white cells) in suspension.

Oker Blum [1] found that the resistance of serum derived from coagulated blood was regularly greater than that derived from defibrinated blood. G. N. Stewart [2] determined that the resistance of defibrinated blood is 3–5 times greater than that of serum; also that the settling of the blood corpuscles in defibrinated blood increases the resistance of the lower layers, because the corpuscles act as foreign, non-electrolytic bodies in suspension (Oker Blum).[3] This increase, due to settling of the corpuscles, can be neglected if the observations occupy but a short time.

The first step of the writer's research was to determine whether during the process of coagulation any change of conductivity would result, especially if the clot were left *in situ*. It might be expected that the change from the colloidal fibrinogen to the solid fibrin would be accompanied by a change in conductivity. What form this change might take could not be predicted, as several as yet unstudied factors require consideration.

As already stated, fibrinogen would act as a partial insulator. Actual experiment alone could show whether changing this substance into fibrin would increase the conductivity, or whether the new-formed fibrin, acting as a solid, would in its turn offer a greater obstruction to the current, for the above-mentioned results of Stewart and Oker Blum throw no light upon this. The small amount of calcium regularly removed from the solution (probably carried down mechanically) and incorporated in the clot, would likewise tend to increase the electrical resistance by reducing the amount of electrolytic substance in solution.

The few investigations at all resembling those to be described are those of Van der Laan,[4] dealing with the changes occurring in milk through acidification, or those of Buchinger,[5] which deal with similar changes brought about by pepsin, in which again the forma-

[1] Oker Blum: See Hamburger, Osmotischer Druck und Ionen-Lehre, 1904, i, p. 477.

[2] G. N. Stewart: Journal of the Boston Society of Medical Sciences, 1897, i, No. 16, p. 18.

[3] Oker Blum: Archiv für die gesammte Physiologie, 1900, lxxix, p. 501.

[4] Van der Laan: Inaugural dissertation, Utrecht, 1896.

[5] Buchinger: Inaugural dissertation, Giessen, 1902.

tion of acids must be reckoned with; and those of Bredig[1] and Lottermoser,[2] etc., who devised the preparation and studied the properties (among them the conductivity) of the metallic colloids and their insoluble hydrosols. Their results cannot be transferred or used in this connection.

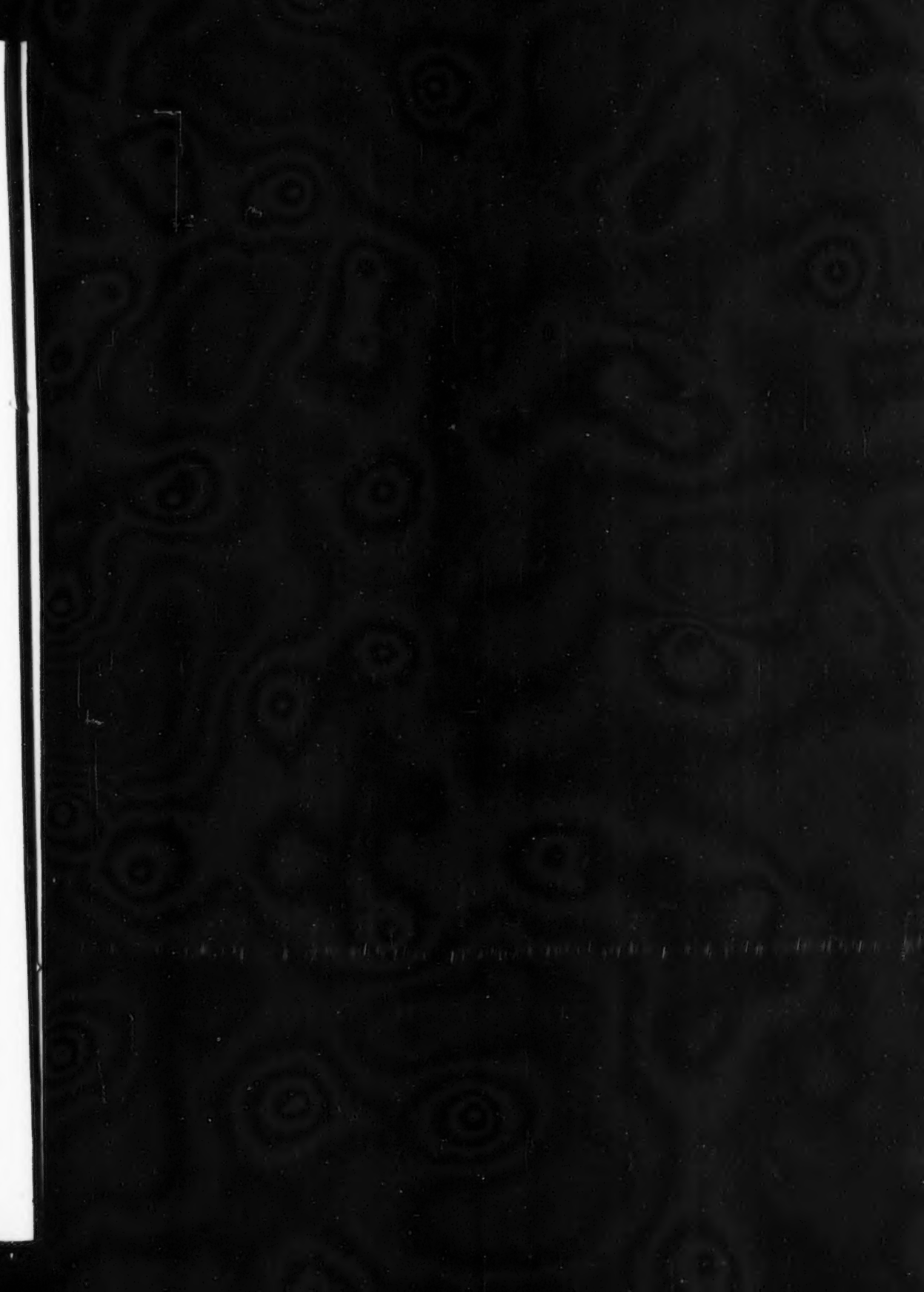
In the writer's experiments the electrical conductivity was determined by the method of Kohlrausch[3] and his smaller apparatus, with sliding bridge, was employed. Freshly drawn blood was caught in small vessels, paraffined to retard coagulation, and kept at constant body temperature until clotting was completed. The conductivity was determined at frequent intervals during the entire process. Electrodes of 2–3 sq. cm. surface, heavily platinized, were used. They were separated between 1–2 cm., depending upon the amount of blood obtainable.

The result showed that there *was no constant or appreciable change during coagulation.* This held good when the electrodes were placed at the uppermost level of the fluid or lower, where the well-formed clot interposed. The fundamental points of the study having thus proven negative, further experiments were not undertaken.

Whether the amount of fibrinogen in blood is too small to show recordable changes with the apparatus used, or whether (which is unlikely) the numerous chemical and physical changes occurring in the complex process of coagulation, exactly neutralize each other, could not be determined.

It might prove of interest to try similar experiments with simpler fluids containing fibrinogen in relatively larger quantities, such as hydrocele fluid or concentrated, artificially prepared solutions of fibrinogen.

[1] BREDIG: Anorganische Fermente, Leipzig, 1901.

[2] LOTTERMOSER: Über anorganische Colloide, Stuttgart, 1901.

[3] KOHLRAUSCH und HOLBORN: Das Leitvermögen der Elektrolyte insbesondere der Lösungen, Leipzig, 1898.